Colloids and Surfaces B: Biointerfaces 125 (2015) 51–57

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Controllable promotion of chondrocyte adhesion and growth on PVA hydrogels by controlled release of TGF-β1 from porous PLGA microspheres

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ARTICLE INFO

Article history:
Received 27 May 2014
Received in revised form 17 October 2014
Accepted 10 November 2014
Available online 15 November 2014

Keywords:
Porous microspheres
Controlled release
Hydrogels
Chondrocyte adhesion
Growth factor

ABSTRACT

Poly(vinyl alcohol) (PVA) hydrogels have been candidate materials for cartilage tissue engineering. However, the cell non-adhesive nature of PVA hydrogels has been a limit. In this paper, the cell adhesion and growth on PVA hydrogels were promoted by compositing with transform growth factor-β1 (TGF-β1) loaded porous poly(D,L-lactide-co-glycolide) (PLGA) microspheres. The porous microspheres were fabricated by a modified double emulsion method with bovine serum albumin (BSA) as porogen. The average pore size of microspheres was manipulated by changing the BSA/PLGA ratio. Such controllable porous structures effectively influenced the encapsulation efficiency (E_{encaps}) and release profile of TGF-β1. By compositing PVA hydrogels with such TGF-β1-loaded PLGA microspheres, chondrocyte adhesion and proliferation were significantly promoted in a controllable manner, as confirmed by fluorescent imaging and quantitative CCK-8 assay. That is, the chondrocyte proliferation was favored by using PLGA microspheres with high E_{encaps} of TGF-β1 or by increasing the PLGA microsphere content in the hydrogels. These results demonstrated a facile method to improve the cell adhesion and growth on the intrinsically cell non-adhesive PVA hydrogels, which may find applications in cartilage substitution.

1. Introduction

Tissue engineering has been promising in treatment of diseased or damaged tissues [1,2], involving delicate combination of scaffolds with cells [3] and bioactive species [4]. PVA hydrogel has been widely studied for cartilage replacement due to its similarities to cartilage in terms of fluid flow, permeability, frictional and mechanical properties [5]. However, PVA hydrogel is in lack of adequate activity to support cell adhesion and growth in comparison to natural extracellular matrix (ECM). The utilization of bioactive substances including proteins or growth factors to combine with scaffolds could stimulate cell differentiation and proliferation, which is critical for tissue regeneration [6,7]. The controlled release of bioactive species (*e.g.*, insulin-like growth factor-1 (IGF-1) [8], TGF-β1 [9] or bone morphogenetic protein-2 (BMP-2) [10]) from hydrogels has been widely utilized to stimulate and regulate cell proliferation and differentiation for tissue engineering. As many biofunctional substances are susceptible to enzymatic degradation or to be captured by non-target organs and tissues, delivery vehicles are needed to protect and convey these bioactive species [11]. Therefore, the encapsulation and controlled release of biofunctional species had been extensively investigated and demonstrated effective in promoting the proliferation of stem cells and regeneration of cartilaginous tissues[12,13].

Polymer microspheres have been widely exploited for protein or growth factor storage and delivery [14] because of their outstanding encapsulation efficiency, controlled release, and regulable degradation [15]. Growth factors encapsulated with polymer microspheres have been demonstrated effective in rendering excellent local promotion of cell adhesion, proliferation, and growth [4,8,16,17]. Jeon et al. [18] have fabricated PLGA microspheres loaded with BMP-2 to induce the differentiation of human adipose-derived stem cells into osteoblasts. The prolonged release of BMP-2 from microspheres was favorable to the formation of mature bone tissue. Moreover, growth factor-loaded microspheres have been combined with hydrogels in order to achieve controlled release

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http://dx.doi.org/10.1016/j.colsurfb.2014.11.010
0927-7765/

and sustained promotion of chondrogenesis of mesenchymal stem cells [9]. Galeska et al. have prepared PLGA microspheres embedded within polyacid-containing PVA hydrogels to control the release of dexamethasone. Polyacids were incorporated to accelerate the PLGA erosion and hence increased the zero-order drug release [19]. Liu et al. have prepared PLGA nanoparticles embedded within PVA hydrogels to reduce the release rate and total amount of insulin [20]. Park et al. have encapsulated TGF-β1 to gelatin microspheres, which were composited with oligo(poly(ethylene glycol) fumarate) hydrogels to upregulate cartilage-relevant genes [21].

In hydrogels composited with growth factor-loaded microspheres, the cell adhesion and proliferation was mainly influenced by the release profile of growth factor, which was controlled by manipulating microsphere structures [22]. It has been a consensus to suppress the burst release of growth factors and to achieve sustained release in order to promote cell proliferation [23]. Lee et al. [24] have reduced the initial burst of vascular endothelial growth factor (VEGF) by using porous PLGA microspheres. By fabricating core–shell alginate/chitosan-polycaprolactone microspheres, the sustained release of VEGF *in vitro* over two weeks has been demonstrated beneficial to cell growth and differentiation [25]. Recently, we have prepared hollow porous PLGA microspheres for controlling BSA release to promote cell adhesion on PVA hydrogels [26]. However, those hollow microspheres provided initial burst release of biomolecules, which was not preferable to achieve sustained release of biomolecules. Besides, it is unclear how the release profile affects the cell adhesion and growth on the composite hydrogels.

In this study, TGF-β1-loaded PLGA microspheres with controllable porous structures were fabricated by a modified double emulsion method. The TGF-β1 release profiles of these microspheres were controlled through manipulation of the particle size, pore dimension, and the TGF-β1 encapsulation efficiency. By compositing these TGF-β1-loaded microspheres with PVA hydrogels, the chondrocyte adhesion and growth on the composite hydrogels were investigated by fluorescent microscopy, scanning electron microscopy, and CCK-8 assay.

2. Materials and methods

2.1. Materials

Poly(D,L-lactide-co-glycolide)OH (M_w = 50,000; 0.35–0.45 dL/g; lactide: glycolide molar ratio 1:1) was purchased from Dai Gang Biology Co., Ltd. Polyvinyl alcohol (PVA, degree of polymerization: 1750 ± 50) was obtained from Sinopharm Chemical Reagent Co., Ltd. Bovine serum albumin (BSA, Yancheng Saibao Biotechnology Company), TGF-β1 (Peportech Co., Ltd.), primary human chondrocytes (ATCC, CHON-001) and all other chemical reagents (A.R.) were used as received.

2.2. Preparation of non-porous and porous PLGA microspheres

Porous PLGA microspheres were prepared by using a modified water-in-oil-in-water (w/o/w) double emulsion method, which induces an osmotic phenomenon, following a protocol previously described [24]. Briefly, 150 mg PLGA was dissolved in 3 ml methylene chloride. BSA (the BSA/PLGA weight ratio was 0.2, 0.4, 0.6 or 0.8) was dissolved in 0.6 ml deionized water. Both solutions were mixed and emulsified by using a QWL500C1Y homogenizer (Quanjian Mechanical and Electrical Co. Ltd., Shanghai, China) for 60 s in an ice bath. A 4% aqueous PVA solution (15 ml) was poured into the resultant emulsion (w/o), followed by emulsification into a double emulsion (w/o/w) under mechanical stirring at 700, 800 or 900 rpm for 5 min at room temperature. The double emulsion was poured into a 0.4% aqueous PVA solution (300 ml), which was mechanically stirred for 4 h to evaporate the organic solvent at 37 °C. The precipitated microspheres were washed with deionized water five times and lyophilized. Non-porous PLGA microspheres were also prepared and used as a control [17].

The microspheres were dispersed in deionized water under ultrasonic agitation for analysis by using a S3500-special Microtrac Particle Size Analyzer (Microtrac, USA) to determine the average size and size distribution. The PLGA microspheres were sputter-coated with a thin gold layer and scanned by using a Hitachi TM-1000 scanning electron microscope (SEM, Tokyo, Japan) at 15 kV. The average pore size of microspheres was determined by analyzing the SEM images by using the Image-Pro Plus Software (Media Cybernetics). At least fifty pores were randomly sampled to take an average of the pore size.

2.3. Preparation of TGF-β1-loaded porous PLGA microspheres

The TGF-β1-loaded porous PLGA microspheres were prepared by dissolving TGF-β1 (1.67 μg/ml) and BSA as porogen in the internal water phase for the double emulsion procedure described in Section 2.2. The TGF-β1-loaded microspheres were stored at −20 °C for futher use. The amount of TGF-β1 encapsulated in the microspheres (M_{encaps}) was calculated by subtracting the TGF-β1 amount in the solution after emulsification ($M_{residual}$) from the total feed amount (M_{feed}). Thus, the encapsulated TGF-β1 amount in the microspheres is given as:

$$M_{encaps} = M_{feed} - M_{residual}$$

where the M_{feed} and $M_{residual}$ values in the solutions were determined by using a TGF-β1 ELISA kit.

The TGF-β1 encapsulation efficiency (E_{encaps}) is given as:

$$E_{encaps}(\%) = \frac{M_{encaps}}{M_{feed}} \times 100\%$$

The E_{encaps} value of BSA was investigated as control by using a BCA protein assay.

2.4. Preparation of microsphere/hydrogel composites

Aqueous PVA solutions of 10 wt% were prepared at 90 °C and cooled down to room temperature for the preparation of hydrogels in all experiments. The PLGA microspheres were added to PVA solutions under magnetic stirring at 37 °C to make a homogeneous dispersion (microsphere/PVA × 100% = 1 wt%, 5 wt%, and 10 wt%). The mixture was injected into a 48-well plate (1.5 mL per well) and frozen at −20 °C for 16 h, followed by thawing for 8 h at 37 °C. Six freeze–thawing cycles were applied to produce microsphere-composited hydrogels with optimal mechanical strength [26].

2.5. Controlled release of TGF-β1

The TGF-β1 release was conducted by incubating a suspension of the TGF-β1-loaded PLGA microspheres (20 mg microspheres in 1 mL PBS) on a water bath at 37 °C for up to 10 days. At predetermined time points, the suspensions were centrifuged and the PBS solution was collected and replaced with fresh PBS. The released TGF-β1 amount was determined by using a TGF-β1 ELISA kit, with the absorbance at 450 nm determined by using a microplate reader. Five specimens were tested for each group. The BSA-loaded microspheres were used as control.