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The enhancement of osteogenesis by nano-fibrous scaffolds incorporating rhBMP-7 nanospheres

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Abstract

It is advantageous to incorporate controlled growth factor delivery into tissue engineering strategies. The objective of this study was to develop a three-dimensional (3D) porous tissue engineering scaffold with the capability of controlled releasing recombinant human bone morphogenetic protein-7 (rhBMP-7) for enhancement of bone regeneration. RhBMP-7 was first encapsulated into poly(lactic-*co*-glycolic acid) (PLGA) nanospheres (NS) with an average diameter of 300 nm. Poly(L-lactic acid) (PLLA) scaffolds with interconnected macroporous and nano-fibrous architectures were prepared using a combined sugar sphere template leaching and phase separation technique. A post-seeding technique was then utilized to immobilize rhBMP-7 containing PLGA nanospheres onto prefabricated nano-fibrous PLLA scaffolds with well-maintained 3D structures. *In vitro* release kinetics indicated that nanosphere immobilized scaffold (NS-scaffold) could release rhBMP-7 in a temporally controlled manner, depending on the chemical and degradation properties of the NS which were immobilized onto the scaffold. *In vivo*, rhBMP-7 delivered from NS-scaffolds induced significant ectopic bone formation throughout the scaffold while passive adsorption of rhBMP-7 into the scaffold resulted in failure of bone induction due to either the loss of rhBMP-7 biological function or insufficient duration within the scaffold. We conclude that the interconnected macroporous architecture and the sustained, prolonged delivery of bioactive rhBMP-7 from NS immobilized nano-fibrous scaffolds actively induced new bone formation throughout the scaffold. The approach offers a new delivery method of BMPs and a novel scaffold design for bone regeneration.

Keywords: BMP; Controlled delivery; Nano fiber; Scaffold; Tissue engineering; Nanospheres

1. Introduction

Bone morphogenetic proteins (BMPs) play an essential role in bone development and regeneration [1,2]. They have been demonstrated to elicit new bone formation both at orthotopic and ectopic sites in experimental animal models [3–7]. Because of their osteogenic potentials [8], recombinant BMPs hold great promise for healing bone fractures, bridging bone nonunions, preventing osteoporosis, and treating periodontal defects. The use of BMP-7 (also known as osteogenic protein 1, OP-1) promotes periodontal regeneration, not only for osteogenesis, but also for cementogenesis and periodontal ligament reconstruction [9–11]. With the validation of the efficacy and safety for bone repair [12–15], recombinant human BMP-2 and 7 (rhBMP-2 and rhBMP-7) have recently obtained FDA approval for human clinical applications to stimulate spinal fusion (InFUSE® Bone Graft) and repair nonunion long bone defect (OP-1™ Implant), respectively.

Unfortunately, exogenous administration of BMPs in buffer solution does not always insure satisfactory new bone induction, especially in higher mammals [16,17]. The

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major reasons are the rapid diffusion of BMPs away from application site and the loss of bioactivity, which lead to insufficient local induction and hence incomplete or failure of bone regeneration. Consequently, it is highly desired to develop an appropriate BMP delivery system to enable a prolonged duration, to reduce BMP proteolysis, denaturation and bioactivity loss and thereby to accelerate tissue healing and regeneration [18–20]. Delivery of BMPs from collagen matrices, while successful in preclinical and human clinical trials [15,21,22], demonstrate a number of disadvantages. It is still difficult to retain the BMPs formulated in a collagen matrix for a sufficient duration, which may explain the great loading and response variability *in vivo* [23]. In addition, the biodegradability and three-dimensional (3D) structures of collagen matrix are difficult to control. Since BMPs are physically entrapped within collagen [24,25], the capability of control over release kinetics from collagen matrix was limited and therefore collagen may not be appropriate for applications where varying release rate is needed. In addition, there are concerns over collagen in terms of immunogenicity and disease transmission [26,27].

BMPs have also been delivered from porous hydroxyapatite (HAP) [28,29], poly(L-lactic acid) (PLLA) [7,30], poly(lactic-*co*-glycolic acid) (PLGA) [31,32] for bone regeneration. However, these carriers have achieved no better success than collagen matrix and none of them has gained acceptance for human clinical investigation. Therefore, it remains a challenge to design an effective tissue engineering scaffold that adequately immobilizes BMPs, temporally and spatially controls release, presents interconnected porosity for vascularization and new bone induction, and ultimately degrades without soliciting unexpected side effects.

Previously, we have encapsulated bioactive polypeptides and proteins into biodegradable nanospheres (NS) for controlled delivery. Recombinant human parathyroid hormone (rhPTH) and platelet-derived growth factor (rhPDGF-BB) were successfully delivered from PLGA NS in a well-controlled pattern and were demonstrated bioactive to stimulate cellular activity [33,34]. Notably, we have immobilized rhPDGF containing PLGA NS onto a 3D PLLA scaffold from which rhPDGF was locally released with adjustable rates [34]. In this paper, we examined the release kinetics of rhBMP-7 from three types of PLGA NS immobilized on a nano-fibrous PLLA scaffold *in vitro* and ectopic bone formation *in vivo*. The rhBMP-7 nanosphere incorporated scaffold offers both osteoinductivity and osteoconductivity for bone tissue engineering.

2. Materials and methods

2.1. Materials

RhBMP-7 was kindly provided by Stryker Biotech (Hopkinton, MA). Iodination of rhBMP-7 (^{125}I-rhBMP-7) was carried out in the Assays and Reagent Facility (Department of Epidemiology) at the University of Michigan. PLGA copolymers with LA/GA ratio of 50:50 (Medisorb®, PLGA50-6.5K, $M_w = 6.5$ kDa; PLGA50-64 K, Mw = 64 kDa) and 75:25 (Medisorb®, PLGA75-113K, Mw = 113 kDa) were purchased from Alkermes Inc. (Wilmington, OH). Poly(L-lactic acid) (PLLA) with inherent viscosity of 1.6 dl/g was purchased from Boehringer Ingelheim (Ingelheim, Germany). Other chemicals used were: poly(vinyl alcohol) (PVA) (88 mol% hydrolyzed, Mw = 25,000) obtained from Polysciences Inc. (Warrington, PA); trifluoroacetic acid (TFA), bovine serum albumin (BSA, Fraction V) and gelatin (type B from bovine skin) from Sigma (St. Louis, MO); dichloromethane, cyclohexane, hexane and tetrahydrofuran from Aldrich Chemical Company (Milwaukee, WI).

2.2. Preparation of nanosphere-immobilized nano-fibrous scaffolds (NS-scaffold)

Lyophilized rhBMP-7 powder was dissolved in 0.1% TFA with 0.1 wt% gelatin and BSA to form a clear aqueous solution. Three PLGA formulations: PLGA50-6.5K, PLGA50-64K and PLGA75-113K were used to encapsulate rhBMP-7 into NS utilizing a double emulsion technique as described previously [33,34]. For release kinetics evaluation, radio-labeled I^{125}-rhBMP-7 was added during nanosphere preparation as a tracer (I^{125}-rhBMP-7: unlabeled rhBMP-7 = 1:100, total 100 ng rhBMP-7 per mg polymer). RhBMP-7 (5 μg/mg polymer) was encapsulated into PLGA50-64K NS for *in vivo* study. Gelatin/BSA-containing PLGA NS (blank NS) were prepared as controls. Macroporous and nano-fibrous PLLA scaffolds were fabricated by the combination of phase separation and sugar-leaching techniques [35]. Highly porous scaffolds were cut into circular disks with dimensions of 7.2 mm in diameter and 2 mm in thickness. The scaffolds were sterilized using ethylene oxide (following the manufacturer's protocol) for 24 h before the BMP NS were immobilized.

PLGA NS were immobilized onto nano-fibrous PLLA scaffolds using a post-seeding method [34]. Briefly, PLGA nanosphere suspension was seeded onto the prefabricated nano-fibrous PLLA scaffold and the scaffold was left in air to evaporate the solvent followed by vacuum drying. The protein amount in a scaffold was modulated by the concentration of protein encapsulated in NS and/or the amount of NS incorporated into the scaffold. The NS-scaffolds were then subjected to a mixed solvent of hexane/THF (volume ratio of 90/10) to immobilize the NS on the scaffold followed by vacuum-drying for 3 days to remove the solvent. NS containing gelatin/BSA were also immobilized onto scaffolds for morphological examination and as controls for release kinetics and animal implantation studies. The morphology of the scaffolds before and after nanosphere immobilization was examined using scanning electron microscopy (SEM, Philips XL30 FEG).

2.3. In vitro release study

RhBMP-7 release profiles from PLGA nanosphere-immobilized PLLA scaffolds were determined *in vitro* by radioactivity detection. One NS-scaffold was placed in 1.0 ml phosphate buffered saline (PBS, 10 mM, pH = 7.4 with 0.1% BSA) at 37 °C under orbital shaking at 60 rpm. Supernatant was collected and equal amount of fresh medium was added to each sample at the designated time points: 1, 3, 5, 7, 10, 14, 21, 28, 35, 42, 49, 56 days for PLGA50-6.5 K NS-scaffolds; 1, 3, 5, 7, 10, 14, 21, 28, 35, 42, 49, 56, 63, 70 days for PLGA50-64K NS-scaffolds; and 1, 3, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70 days for PLGA75-113K NS-scaffolds. The radioactivity of collected supernatant was analyzed using a gamma counter (Gamma 5500, Beckman) and converted to calculate the quantity of the released rhBMP-7. Scaffolds with gelatin/BSA containing NS were used as controls.

2.4. Preparation of implants

Three groups of scaffold implants were prepared for *in vivo* study on rats as listed in Table 1. Group I (Control scaffolds): PLLA scaffold with PLGA50-64K NS containing gelatin/BSA; Group II (rhBMP-7 adsorbed