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Preparation of dexamethasone-loaded biphasic calcium phosphate nanoparticles/collagen porous composite scaffolds for bone tissue engineering

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ABSTRACT

Although bone is regenerative, its regeneration capacity is limited. For bone defects beyond a critical size, further intervention is required. As an attractive strategy, bone tissue engineering (bone TE) has been widely investigated to repair bone defects. However, the rapid and effective bone regeneration of large non-healing defects is still a great challenge. Multifunctional scaffolds having osteoinductivity and osteoconductivity are desirable to fasten functional bone tissue regeneration. In the present study, biomimetic composite scaffolds of collagen and biphasic calcium phosphate nanoparticles (BCP NPs) with a controlled release of dexamethasone (DEX) and the controlled pore structures were prepared for bone TE. DEX was introduced in the BCP NPs during preparation of the BCP NPs and hybridized with collagen scaffolds, which pore structures were controlled by using pre-prepared ice particulates as a porogen material. The composite scaffolds had well controlled and interconnected pore structures, high mechanical strength and a sustained release of DEX. The composite scaffolds showed good biocompatibility and promoted osteogenic differentiation of hMSCs when used for three-dimensional culture of human bone marrow-derived mesenchymal stem cells. Subcutaneous implantation of the composite scaffolds at the dorsa of athymic nude mice demonstrated that they facilitated the ectopic bone tissue regeneration. The results indicated the DEX-loaded BCP NPs/collagen composite scaffolds had high potential for bone TE.

Statement of Significance

Scaffolds play a crucial role for regeneration of large bone defects. Biomimetic scaffolds having the same composition of natural bone and a controlled release of osteoinductive factors are desirable for promotion of bone regeneration. In this study, composite scaffolds of collagen and biphasic CaP nanoparticles (BCP NPs) with a controlled release nature of dexamethasone (DEX) were prepared and their porous structures were controlled by using ice particulates. *In vitro* cell culture and *in vivo* implantation experiments demonstrated the composite scaffolds exerted synergistic effects on the osteogenic differentiation of hMSCs and bone regeneration. The composite scaffolds also showed promotive effect on the formation of capillary blood vessels in the regenerated bone. This study is the first research to prepare DEX-loaded BCP NPs/collagen porous composite scaffolds. The superior performance of the composite scaffolds indicates the composite scaffolds should be useful for bone tissue engineering.

1. Introduction

Bone defect is one of the diseases most threatening human health. Although bone is regenerative, its regeneration capacity is limited. Further intervention is necessary for the bone defects beyond a critical size. Nowadays, autografts and allografts are still the most common solutions in clinical applications. Nevertheless, they have some drawbacks such as the limited sources, extra invasive surgery and immunological rejection [1]. Therefore, more and more attention has been drawn to bone tissue engineering (bone TE). Bone TE is a strategy combining biotechnology and biomaterials to induce new bone regeneration, which is a complex and dynamic process that starts with cell migration and adhesion

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followed by cell proliferation, differentiation, matrix formation along with remodeling of bone [2]. Scaffolds have been used for bone TE to control cell functions and keep regeneration spaces. Besides good biocompatibility and biodegradability, scaffolds used for bone TE should have excellent osteoconductivity, osteoinductivity, interconnected porous structure and high mechanical property [3–5]. Osteoconductivity can ensure migration, adhesion and survival of osteogenic cells. Osteoinductivity can induce osteogenic differentiation of stem cells [6].

Many efforts have been made to prepare scaffolds for bone TE. To mimic the extracellular matrix composition of bone, a variety of strategies have been considered, including usage of the components present in natural bone [7], controlling of pore structure and interconnectivity [8], construction of multiple scale architectures [9] and incorporation of growth factors [10]. For example, calcium phosphate (CaP) has been hybridized with polymers in the forms of tablets [11], blends [12], pastes [13] or cements [14]. However, these CaP/polymer composite scaffolds do not have appropriate pore structures for cell accommodation and migration. In particular, their pore interconnectivity is poor [15–17].

To render scaffolds with good osteoinductivity, growth factors have been incorporated in the scaffolds. Nevertheless, the most commonly used growth factors such as bone morphogenetic proteins (BMPs) and transforming growth factor beta (TGF-β) are proteins and can easily lose their bioactivity during the preparation procedures [18]. To minimize denaturation and maintain their bioactivity, growth factors are generally introduced in scaffolds by physical adsorption. The release of physically adsorbed proteins always exhibits an initial burst and cannot last for a long period [19–24]. On the other hand, dexamethasone (DEX), as a low molecular weight osteoinductive factor, has drawn much attention for incorporation in scaffolds for bone TE because of its high stability even in tough chemical environment [25]. DEX and hydroxyapatite nanoparticles have been hybridized with gelatin and poly(L-lactide) to construct hydroxyapatite/DEX/PLLA/gelatin composite scaffold by electrospinning technique [26]. DEX-loaded mesoporous silica nanoparticles have been deposited onto poly(L-lactic acid)/poly(ε–caprolactone) nanofibrous scaffold by electrophoretic deposition [27]. A dual delivery system of BMP-2 and DEX has been designed by co-electrospinning the blending solution that is composed of BMP-2-encapsulated bovine serum albumin nanoparticles, DEX and poly(ε-caprolactone)-co-poly(ethylene glycol) (PCE) copolymer [28]. These composite scaffolds have shown the synergistic effects of the released osteogenic factors. However, the problems of initial burst release and a short release period of DEX need to be solved. Furthermore, simultaneous release of both DEX and calcium and phosphorus ions is desirable for synergistic promotion of new bone regeneration.

In this study, composite scaffolds of collagen and DEX-loaded biphasic CaP nanoparticles (BCP NPs) were prepared for a sustainable release of DEX together with the calcium and phosphorous ions. DEX was incorporated in the BCP NPs during their preparation to allow the DEX being physically locked in the crystals of BCP NPs for a sustainable and simultaneous release profile of DEX, calcium and phosphorous ions. The microporous structure of the composite scaffolds was controlled by using pre-prepared ice particulates as a porogen material. The composite scaffolds were used for three-dimensional culture of human bone marrow-derived mesenchymal stem cells (hMSCs). Their effects on proliferation and osteogenic differentiation of hMSCs were compared with collagen scaffold and BCP NPs/collagen composite scaffold. Subcutaneous implantation of the composite scaffolds at the dorsa of athymic nude mice was used to demonstrate their promotive effects on ectopic bone tissue regeneration.

2. Materials and methods

2.1. Preparation of BCP NPs and DEX@BCP NPs

Biphasic calcium phosphate nanoparticles (BCP NPs) were prepared by adding dropwise 75.0 mL of 0.5 M calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$, Sigma-Aldrich, USA) solution to 50 mL of 0.5 M ammonium phosphate dibasic ($(NH_4)_2HPO_4$, Sigma-Aldrich, USA)) solution by a syringe pump (KD Scientific Inc., USA) [29]. After reacting at 55 °C and a pH of 9.5 for 30 min under stirring (700 rpm), the slurry was aged for 36 h at room temperature to form stable BCP NPs. DEX-loaded BCP NPs (denoted as DEX@BCP NPs) were prepared by adding dropwise the mixture solution of DEX and $Ca(NO_3)_2{\cdot}4H_2O$ (75.0 mL) to 50 mL of 0.5 M $(NH_4)_2HPO_4$ solution. The mixture solution of DEX and $Ca(NO_3)_2{\cdot}4H_2O$ was prepared by adding 3 mL of 0.75, 1.50 and 2.25 mg/mL of DEX in ethanol to 72.0 mL of 0.52 M $Ca(NO_3)_2{\cdot}4H_2O$ solution. The reaction was continued in a 55 °C water bath for 30 min under stirring and then aged for 36 h at room temperature to obtain stable DEX@BCP NPs. The DEX@BCP NPs prepared at the three different DEX feeding concentrations were denoted as DEX1@BCP NPs, DEX2@BCP NPs and DEX3@BCP NPs, respectively. The prepared DEX@BCP NPs were dispersed in 20 mL ethanol and shaken at 200 rpm and room temperature for 20 min followed with centrifugation at 8000 rpm. The washing with ethanol was repeated for 3 times to remove the DEX adsorbed on the surface of the DEX@BCP NPs.

2.2. Preparation of DEX@BCP NPs/collagen composite scaffolds

A 2.5% (w/v) solution of collagen was prepared by dissolving porcine type I collagen (Nitta Gelatin, Japan) in a 10% (v/v) ethanol aqueous solution. Ice particulates were prepared by spaying pure water in liquid nitrogen and ice particulates with the diameters between 425 and 500 μm were obtained by sieving the ice particulates through two sieves having a respective mesh size of 425 and 500 μm [30]. The BCP NPs, DEX1@BCP NPs, DEX2@BCP NPs and DEX3@BCP NPs were individually dispersed in a 10% (v/v) ethanol aqueous solution to prepare their respective dispersion solution. The collagen solution, NPs dispersion solution and ice particulates were kept in a −5 °C low temperature chamber (Espec, Osaka, Japan) for 2 h to balance their temperature to −5 °C.

To optimize the mass ratio of NPs and collagen in the composite scaffolds, a series of porous BCP NPs/collagen (BCP/Col) composite scaffolds with a different mass ratio of BCP NPs/collagen were prepared. At first, 3 mL of the pre-cooled BCP NPs suspension solution at a concentration of 187, 140, 112 and 93 mg/mL was added dropwise to 11 mL of the pre-cooled collagen solution (2.5% (w/v)) at −5 °C and mixed well. The final concentration of collagen in the BCP NPs/collagen suspension solution was 2.0% (w/v). The mass ratio of the BCP NPs/collagen was 2:1, 1.5:1, 1.2:1 and 1:1 (w/w), respectively. Subsequently, the temperature-balanced ice particulates were added to the BCP NPs/collagen suspension solution at a ratio of 50:50 (w/v) at −5 °C. The components were mixed carefully to allow the ice particulates being homogeneously distributed in the collagen/BCP NPs suspension solution without air bubble generation and then poured into silicone frames which were placed on PFA film-wrapped copper plates. The entire constructs were placed at −12 °C for 12 h to slowly freeze the mixture solution and then frozen at −80 °C for 6 h. Finally, the frozen constructs were freeze-dried for 2 days in a Wizard 2.0 freeze dryer (VirTis, Gardiner, NY). The freeze-dried constructs were cross-linked with 50 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, Peptide Institute, Inc.) and 20 mM N-hydroxysuccimide (NHS, Wako Pure Chemical Industries, Ltd.) in an 80% (v/v) ethanol