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Therapeutic-designed electrospun bone scaffolds: Mesoporous bioactive nanocarriers in hollow fiber composites to sequentially deliver dual growth factors

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ABSTRACT

A novel therapeutic design of nanofibrous scaffolds, holding a capacity to load and deliver dual growth factors, that targets bone regeneration is proposed. Mesoporous bioactive glass nanospheres (MBNs) were used as bioactive nanocarriers for long-term delivery of the osteogenic enhancer fibroblast growth factor 18 (FGF18). Furthermore, a core–shell structure of a biopolymer fiber made of polyethylene oxide/polycaprolactone was introduced to load FGF2, another type of cell proliferative and angiogenic growth factor, safely within the core while releasing it more rapidly than FGF18. The prepared MBNs showed enlarged mesopores of about 7 nm, with a large surface area and pore volume. The protein-loading capacity of MBNs was as high as 13% when tested using cytochrome C, a model protein. The protein-loaded MBNs were smoothly incorporated within the core of the fiber by electrospinning, while preserving a fibrous morphology. The incorporation of MBNs significantly increased the apatite-forming ability and mechanical properties of the core–shell fibers. The possibility of sequential delivery of two experimental growth factors, FGF2 and FGF18, incorporated either within the core–shell fiber (FGF2) or within MBNs (FGF18), was demonstrated by the use of cytochrome C. In vitro studies using rat mesenchymal stem cells demonstrated the effects of the FGF2–FGF18 loadings: significant stimulation of cell proliferation as well as the induction of alkaline phosphate activity and cellular mineralization. An in vivo study performed on rat calvarium defects for 6 weeks demonstrated that FGF2–FGF18-loaded fiber scaffolds had significantly higher bone-forming ability, in terms of bone volume and density. The current design utilizing novel MBN nanocarriers with a core–shell structure aims to release two types of growth factors, FGF2 and FGF18, in a sequential manner, and is considered to provide a promising therapeutic scaffold platform that is effective for bone regeneration.

1. Introduction

Therapeutic scaffolds that possess the capacity to load and deliver therapeutically relevant molecules, including drugs, proteins and genes, are considered to be promising platforms for the engineering of tissues, including bone [1]. While the approaches that tailor the scaffolds intrinsically, including hybridization, mineralization and stiffness matching, can also be effective in bone regeneration [2–6], the controlled delivery of extrinsic bioactive molecules provides the scaffolds with additional therapeutic functions. The scaffolds play direct roles in triggering the functions of the stem/progenitor cells by providing physical and biochemical environments for homing and recruiting cells when implanted in vivo [7]. Many methodologies have been developed to load therapeutic molecules effectively onto the scaffolding materials, including surface immobilization, affinity-driven tethering, direct incorporation within the structure and nano/microencapsulation [8–10].

Electrospun fibers have shown great potential as a scaffold platform for the repair and regeneration of a series of tissues, such as skin, muscle, nerve, cartilage and bone [11–14]. The fibrous morphology, which is tunable from hundreds to a few micrometers, largely mimics the native tissue extracellular matrices, thus favoring cellular adhesion and growth [15]. The electrospinning technique enables the formation of fibers with different morphologies, structures and compositions. In particular, for drug

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delivery purposes, fibers with a core–shell structure have shown great promise since relatively large quantities of therapeutic molecules can be encapsulated within the core while preserving the biological activity of the molecules [16,17]. Theoretically, the release of the encapsulated drugs can be controlled by altering the shell composition and thickness.

Here we also focus on this particular type of electrospun core–shell fibrous scaffold, with a view to developing a drug delivering scaffold for bone regeneration. Furthermore, we intend to deliver two different types of therapeutic molecules in a sequential manner. One type of drug will be released relatively quickly, while the other drug is released in a more sustained manner. This concept of sequential delivery has recently gained great interest for scaffold designs targeting tissue repair and regeneration, which generally requires the time-dependent action of multiple molecules [18–21].

We use fibroblast growth factor 2 (FGF2) as the fast-releasing molecule while FGF18 is used as the slow-releasing one, aiming at achieving an effective delivery system for bone regeneration. FGF2 has long been shown to stimulate mitosis and proliferation of cells, including those of fibroblasts and endothelial cells, which play an important role in populating and maintaining such cells for the subsequent tissue repair process [22]. On the other hand, FGF18, a growth factor in the FGF family that has been highlighted relatively more recently, is known to stimulate cellular osteogenesis by the up-regulation of bone morphogenetic protein 2 [23]. Therefore, the co-delivery of both growth factors FGF2 and FGF18 in a sequential release pattern is considered to be effective for the bone repair and regeneration process.

In order to realize sequential delivery, we first preload FGF18 within mesoporous bioactive glass nanospheres (MBNs), which are then encapsulated within the core, where FGF2 is also loaded directly. The MBNs recently developed by our group have shown great performance in loading and delivering therapeutic molecules, including chemical drugs, proteins and genes [24–26]. Furthermore, MBNs are an attractive nanomaterial platform for the regeneration of hard tissues like bone and tooth because the nanospheres have shown outstanding mineralization behavior, which is mainly ascribed to the high surface bioreactivity [18]. Furthermore, the possible release of Ca and Si ions from the nanospheres would be beneficial for stimulating progenitor and stem cells into an osteogenic lineage [27].

Fig. 1 illustrates the schematic design of the present study, the development of a novel therapeutic bone scaffold where FGF18-preloaded MBNs are incorporated within the FGF2-loaded core–shell electrospun polymeric fiber. FGF2 is to be released initially to stimulate cellular mitosis and possible angiogenesis, while FGF18 is to be released more slowly to induce osteogenesis. In this study, we describe the processing tools used for the therapeutic scaffolds and investigate their physico-chemical properties, including the morphological development, mechanical properties, in vitro degradation and bone bioactivity. The release pattern of the growth factors is monitored using a model protein for a long period of a few months. The therapeutic efficacy of the FGF2/FGF18 delivered from the fiber scaffolds is addressed in terms of the in vitro responses of rat mesenchymal stem cells (MSCs), including growth and osteogenic differentiation, as well as in vivo bone formation in rat calvarium defects.

2. Materials and methods

2.1. Mesoporous bioactive glass nanospheres

MBNs were prepared in basic solution at room temperature using water, ethanol and 2-ethoxyethanol as co-solvents and hexadecyltrimethylammonium bromide (CTAB) as a surfactant. In a typical procedure, 1 g of CTAB was dissolved in an emulsion system composed of 150 ml of H_2O, 2 ml of aqueous ammonia, 10 ml of 2-ethoxyethanol, 20 ml of ethanol and tetrahydrate calcium nitrate ($Ca(NO_3)_2{\cdot}4H_2O$). After the mixture had been vigorously stirred for 0.5 h at room temperature, tetraethyl orthosilicate was quickly dripped into the mixture. The molar ratio of Ca:Si was 15:85. The resulting mixture was vigorously stirred at room temperature for 4 h. A white precipitate was obtained, filtered, washed with pure water and dried in air at 60 °C for 24 h. The mixture was then treated with 20 mg of ammonium nitrate solution in 40 ml of deionized water at 60 °C overnight to remove any remaining CTAB. The nanoparticle solution was centrifuged and washed with ethanol and deionized water, then dried overnight under a vacuum to obtain MBNs.

2.2. Electrospinning of core–shell hollow-structured composite fibers

The core–shell hollow-structured fiber was made of polycaprolactone (PCL; Mw = 80,000; Sigma–Aldrich) and poly(ethylene oxide) (PEO; Mw = 60,000; Sigma–Aldrich) as the outer and inner parts. The MBNs were added to the PEG at varying contents (1, 3 and 5 wt.% with respect to PEO). The solvent used for PCL was a co-solvent comprising chloroform and dimethyl formamide mixed at a ratio of 4:1, and that for PEO was distilled water. PCL was dissolved at 10% and PEO was dissolved at 4%. Coaxial electrospinning was conducted to prepare PCL as the shell and PEO (+MBN) as the core using a syringe-like apparatus with an inner needle placed coaxially inside an outer one. The feed rates were 4.0 and 0.5 ml h^{-1}, respectively, for the outer and the inner part. Each solution loaded into the syringe was injected through a spinneret needle under the application of high voltage power (Gamma High Voltage Research, Ormond Beach) onto a slowly rotating metal collector. The distance between the needle and the collector was set to 15 cm, and the collection was carried out over 2 h. The collected fiber mesh was vacuum-dried overnight. In particular, to load the proteins and growth factors within the fiber, the solution, at a concentration 100 μg ml^{-1} in phosphate-buffered saline (PBS), was mixed with the PEO solution, which was adequate for electrospinning.

2.3. Characterizations

The morphology and the nanostructure of the samples were examined respectively by field-emission scanning electron microscopy (SEM; Tescan, Mira II LMH) and high-resolution transmission electron microscopy (TEM; JEM-3010, JEOL, operating at 300 kV). The chemical bond structure was investigated by attenuated total reflectance–Fourier transform infrared spectroscopy (ATR–FTIR) at a resolution of 4 cm^{-1} in the range from 4000 to 400 cm^{-1} using a Varian 640-IR spectroscope with a GladiATR diamond crystal accessory (PIKE Technologies). The thermal behavior of the samples was investigated by thermogravimetric analysis (TGA; TGA N-1500, Scinco, operating at a heating rate of 10 °C min^{-1}). The mesopore structure was analyzed according to N_2 adsorption–desorption measurements using an automated surface area and pore size analyzer (Quadrasorb SI, Quantachrom Instruments). The specific surface area was estimated according to the Brunauer–Emmett–Teller (BET) method, and the pore size distribution was determined using the non-local density functional theory (NLDFT) method. The surface electrical properties were investigated with ξ-potential measurements (Zetasizer Nano ZS, Malvern Instruments), and the ξ-potential was measured in water at 25 °C at a pH of 7 with an applied field strength of 20 V cm^{-1}. The instrument automatically calculates the electrophoretic mobility (U), and the ξ-potential is obtained according to the

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