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Fabrication of a three-dimensional β-tricalcium-phosphate/gelatin containing chitosan-based nanoparticles for sustained release of bone morphogenetic protein-2: Implication for bone tissue engineering

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ABSTRACT

Fabrication of an ideal scaffold having proper composition, physical structure and able to have sustained release of growth factors still is challenging for bone tissue engineering. Current study aimed to design an appropriate three-dimensional (3-D) scaffold with suitable physical characteristics, including proper compressive strength, degradation rate, porosity, and able to sustained release of bone morphogenetic protein-2 (BMP2), for bone tissue engineering. A highly porous 3-D β-tricalcium phosphate (β-TCP) scaffolds, inside of which two perpendicular canals were created, was fabricated using foam-casting technique. Then, scaffolds were coated with gelatin layer. Next, BMP2-loaded chitosan (CS) nanoparticles were dispersed into collagen hydrogel and filled into the scaffold canals. Physical characteristics of fabricated constructs were evaluated. Moreover, the capability of given construct for bone regeneration has been evaluated in vitro in interaction with human buccal fat pad-derived stem cells (hBFPSCs). The results showed that gelatin-coated TCP scaffold with rhBMP2 delivery system not only could act as a mechanically and biologically compatible framework, but also act as an osteoinductive graft by sustained delivering of rhBMP2 in a therapeutic window for differentiation of hBFPSCs towards the osteoblast lineage. The proposed scaffold model can be suggested for delivering of cells and other growth factors such as vascular endothelial growth factor (VEGF), alone or in combination, for future investigations.

1. Introduction

Bone tissue engineering using scaffold can directly influence by both scaffold composition and its physical structure [1]. Apart from being biocompatible, the rate of scaffold degradation should be similar to the rate of new bone formation during which the bone graft would be, eventually, fully replaced by newly formed bone [2]. Also, mechanical properties should be similar to those of the tissue repair site and this property is more important for load-bearing areas. Moreover, the scaffolds should serve as templates to form vascular system, and growth of osteoprogenitor cells. However, three-dimensional (3-D) scaffolds have shown some limitations such as lack of de novo bone tissue growth, inadequate nutrient supply and poor waste removal [3]. Studies showed that 3-D scaffolds are proper for bone regeneration in critical-sized bone defects; however, new bone is slowly formed into the middle of the scaffold [4]. Indeed, the ability to induce and support vascular infiltration is crucial for the efficacy of the scaffolds in order to successful bone regeneration.

Scaffolds also serve as a delivery system for numerous growth factors to the site of bone defects. Growth factors have been shown to control cellular functions and promote bone regeneration during natural healing process [5]. However, limitations including their short half-life and rapid diffusion from the defect area lead to adverse side effects and inefficient tissue formation [6,7] which pose a hamper in their clinical applications. A local delivery system is introduced as a device to carry and discharge therapeutic agents including drugs, growth factors and cells to specific sites for a desired period of time. Local sustained delivery of growth factors inside a 3-D scaffold has been hypothesized to improve bone regeneration even though the production of reliable biopolymer particles has been still remained as a challenge [8].

Delivery systems based on chitosan (CS) have extensively used since they can be able to have sustained release of drugs at a specific local targeted area [9]. Chitosan is a biocompatible polysaccharide obtained from the deacetylation of chitin, a natural polysaccharide in marine crustaceans [10]. Chitosan nanoparticles have gained popularity as a drug delivery carrier for many advantages including low toxicity, better stability, providing versatile routes of administration, and simple and

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mild preparation methods [11,12]. Also, collagen sponges have been used as a clinical carrier of recombinant human bone morphogenetic protein-2 (rhBMP2) [13]. The scaffolds consisted only collagen is not desirable for bone defects regeneration due to its low resistance and early degradation within the body [14] which can lead mainly to a burst release of BMP2. However, resorbability, low antigenicity, cytocompatibility and tissue regeneration potential properties have attracted more attention to using collagen in composite with other biomaterials [15].

Bioactive calcium phosphate ceramics have demonstrated excellent osteoconductivity and biocompatibility for over 30 years study in orthopedics and reconstructive surgeries [16]. β-tricalcium phosphate (β-TCP, β-$Ca_3(PO_4)_2$) is currently one of the most widely used calcium phosphate in bone tissue engineering [17–20]. In fact, β-TCP has displayed osteogenic property, phase stability and strong bond formation with the host bone tissue; moreover, not only β-TCP has a Ca/P ratio similar to that of natural bone tissue, but also it showed progressive biodegradability accompanied by simultaneous formation of normal bone structure in vivo [21]. Nonetheless, the β-TCP scaffolds have a brittle fracture behavior against low stress levels in the clinical applications. The combination of gelatin with β-TCP have been used favorably in bone tissue engineering and studies has been shown that addition of gelatin to β-TCP enhanced its mechanical properties [22,23]. Moreover, gelatin, a partially hydrolyzed form of collagen, provides Arg-Gly-Asp (RGD) motifs which can mediate cell attachment via interaction with integrin; hence, it enhances biocompatibility and osteconductivity of the scaffold [24].

Current study tend to design an appropriate 3-D scaffold with proper physical characteristics and with capability to sustainably release BMP2 for bone tissue engineering. A highly porous 3-D β-TCP scaffold, inside of which two perpendicular canals were created, was fabricated. The mechanical strength of the scaffold was enhanced with gelatin coating layer. In addition, BMP2-loaded CS nanoparticles were dispersed into collagen hydrogel and filled into the scaffold canals. The capability of given construct for bone regeneration has been evaluated in vitro in interaction with buccal fat pad-derived stem cells (BFPSCs). The proposed design with sustained release of growth factor from scaffold center is not only suitable for delivery of BMP2, but also can be proposed for other growth factors including vascular endothelial growth factor (VEGF) for angiogenesis induction.

2. Material and methods

2.1. Fabrication and characterization of highly porous β-TCP scaffolds coated with gelatin (gTCP)

Fig. 1 illustrates the procedures of β-TCP scaffolds fabrication and the outline of experiments. The β-TCP scaffolds in cubic form ($7 \times 7 \times 7$ mm) with two perpendicular canals in diameter of 2 mm were fabricated by foam-casting method using interconnected polyurethane foam (PU) (Safoam, Tehran, Iran) as a template. The PU foam was cut into cubic forms, and two perpendicular canals were created into them using a perforating device. Then, it was cleaned and dried before immersion in the TCP slurry. Ceramic slurry was prepared by dissolving tricalcium phosphate (TCP) (Sigma-Aldrich, St. Louis, Missouri, United States) powder (in ration of 1 g/ml) in distilled water. Colloidal silica (30 wt%), carboxy methyl cellulose (CMC, 0.2 wt%) and sodium tripolyphosphate pentabasic (TPP, 0.5 wt%) (Sigma-Aldrich, St. Louis, Missouri, United States) were added into the slurry to obtain favorable rheological properties. The PU pieces were compressed slightly to improve their absorption. Squeezing the samples caused to removing the excessive slurry and opening the interconnected pores. After drying at room temperature for 24 h, the samples were placed into a furnace according to a strict schedule as follow: the samples were heated from room temperature to 600 °C with a rate of 0.5 °C/min, held at 600 °C for an hour; then, the temperature were raised to 1200 °C with a rate

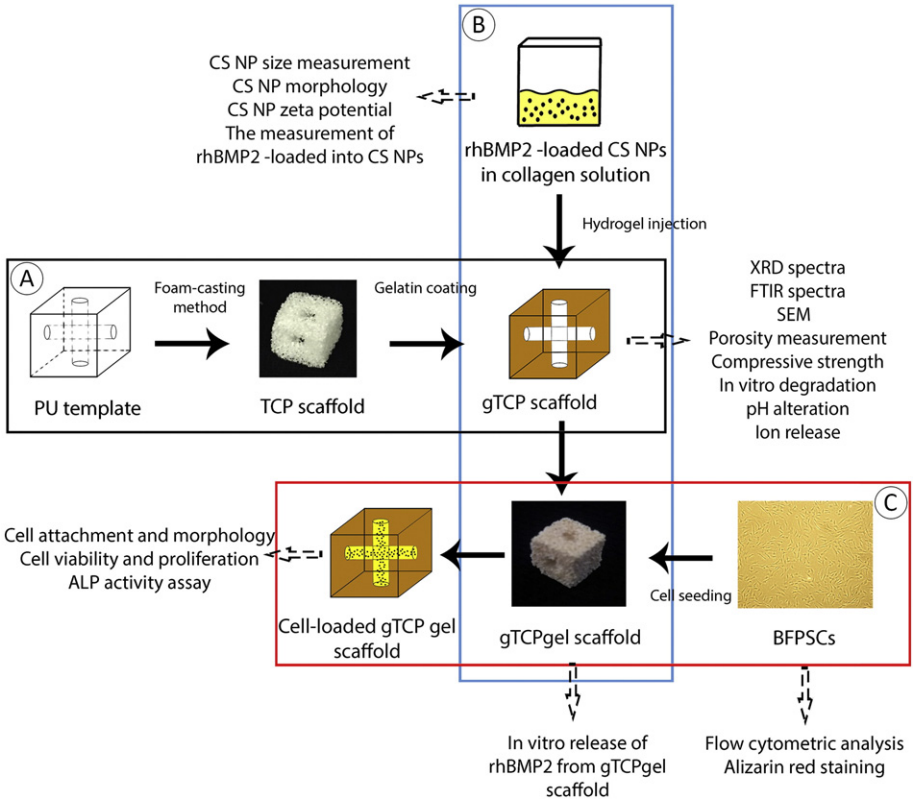

Fig. 1. The flow chart of the study design; (A) Fabrication of the 3-D gTCP scaffold; (B) Fabrication of rhBMP2 delivery system injected into the canals of the gTCP scaffold (gTCPgel); (C) Biologic analyses of the gTCPgel scaffold with sustained release of rhBMP2.