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Direct deposited porous scaffolds of calcium phosphate cement with alginate for drug delivery and bone tissue engineering

Gil-Su Lee [a,b], Jeong-Hui Park [a,b], Ueon Sang Shin [a,b], Hae-Won Kim [a,b,c,*]

[a] Department of Nanobiomedical Science and WCU Research Center, Dankook University Graduate School, Cheonan, South Korea
[b] Institute of Tissue Regeneration Engineering, Dankook University, Cheonan, South Korea
[c] Department of Biomaterials Science, School of Dentistry, Dankook University, Cheonan, South Korea

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ABSTRACT

This study reports the preparation of novel porous scaffolds of calcium phosphate cement (CPC) combined with alginate, and their potential usefulness as a three-dimensional (3-D) matrix for drug delivery and tissue engineering of bone. An α-tricalcium phosphate-based powder was mixed with sodium alginate solution and then directly injected into a fibrous structure in a Ca-containing bath. A rapid hardening reaction of the alginate with Ca^{2+} helps to shape the composite into a fibrous form with diameters of hundreds of micrometers, and subsequent pressing in a mold allows the formation of 3-D porous scaffolds with different porosity levels. After transformation of the CPC into a calcium-deficient hydroxyapatite phase in simulated biological fluid the scaffold was shown to retain its mechanical stability. During the process biological proteins, such as bovine serum albumin and lysozyme, used as model proteins, were observed to be effectively loaded onto and released from the scaffolds for up to more than a month, proving the efficacy of the scaffolds as a drug delivering matrix. Mesenchymal stem cells (MSCs) were isolated from rat bone marrow and then cultured on the CPC–alginate porous scaffolds to investigate the ability to support proliferation of cells and their subsequent differentiation along the osteogenic lineage. It was shown that MSCs increasingly actively populated and also permeated into the porous network with time of culture. In particular, cells cultured within a scaffold with a relatively high porosity level showed favorable proliferation and osteogenic differentiation. An in vivo pilot study of the CPC–alginate porous scaffolds after implantation into the rat calvarium for 6 weeks revealed the formation of new bone tissue within the scaffold, closing the defect almost completely. Based on these results, the newly developed CPC–alginate porous scaffolds could be potentially useful as a 3-D matrix for drug delivery and tissue engineering of bone.

1. Introduction

Rapid setting cements are very useful in bone tissue regeneration, as either a direct filling or an injectable material. Calcium phosphate cements (CPCs) have been one of the most widely studied bioactive ceramics for this purpose [1,2]. Although some challenges, such as the mechanical properties, the introduction of macropores and control of the dissolution rate, remain to be improved, many fascinating properties of CPCs make them a useful choice in the treatment of bone defects [2,3]. CPCs have been found to be cell and tissue compatible, and they self-set, making them useful as an injectable material requiring minimally invasive surgery, and, in addition, they can carry therapeutic molecules within the formulation [4,5].

Scaffolds with a three-dimensional (3-D) porous network provide effective matrix conditions for bone tissue engineering [6–8]. Tissue cells are ex vivo cultured with the scaffolds to better mimic the structure and function of native tissues than the materials or cells alone [8,9]. In the course of ex vivo engineering of tissues the controlled release of therapeutic molecules such as growth factors is favored, to modulate cellular function and speed up bone formation. CPC-based materials have also been considered good candidates for the delivery of therapeutics carried within their structure, because they self-harden under mild conditions, with the therapeutics being safely incorporated, and retain a sustainable release profile [4]. To apply CPC-based materials to bone tissue engineering their development as 3-D scaffolds which support cell proliferation and cell–material composite construction is necessary.

To this end we here aim to develop a novel cell scaffolding material made of CPCs in combination with sodium alginate. In particular, a fibrous network was formulated by directly depositing

* Corresponding author at: Institute of Tissue Regeneration Engineering (ITREN), Dankook University, Cheonan, South Korea. Tel.: +82 41 550 3081.
E-mail address: kimhw@dku.edu (H.-W. Kim).

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the composite suspension under a Ca-containing solution. The deposited suspension rapidly sets to form a gelled network in the presence of alginate and is cross-linked by Ca^{2+} ions [10,11]. The hardened porous scaffold is considered to be cell compatible and useful for bone tissue engineering. Moreover, the scaffold is considered to be able to load and deliver bioactive molecules contained within the structure. The processing techniques to develop the CPC–alginate porous scaffolds are described and the in vitro cellular responses of mesenchymal stem cells (MSCs) from rat bone marrow to them have been investigated, prior to their application in bone tissue engineering. An in vivo pilot study was also performed to evaluate tissue compatibility, and the drug delivery potential of the scaffold was assessed using two different model proteins.

2. Materials and methods

2.1. Preparation of the composite suspension

The experimental α-tricalcium phosphate (α-TCP)-based cement powder was prepared as described in a previous report [12]. Commercial calcium carbonate and anhydrous dicalcium phosphate (both from Aldrich) were mixed and thermally reacted at 1400 °C for 3 h, then air quenched, which resulted in complete reaction to form the α-TCP phase [12]. The powder was ball milled and sieved down to 45 μm, and then kept under vacuum for further use. The average particle size of the α-TCP was 4.79 μm, measured using a particle size analyzer (Saturn DigiSizer 5200, Micromeritics, USA). Sodium alginate (Aldrich) solution was prepared in 5% Na_2HPO_4 (in distilled, deionized water) at a concentration of 2 wt.%. The cement powder was mixed with the alginate solution at an appropriate ratio to prepare the composite suspension for dispensation through a nozzle.

2.2. Direct deposition into scaffolds

The possibility of the direct deposition of the composite suspensions was examined by varying the mixing ratio of cement powder/alginate solution (from 1.0 to 2.5 by weight). Above a ratio of 2.0 the suspension was too viscous to dispense through the nozzle, therefore ratios of 1.0–2.0 were used for the experiments. The mixed suspension was placed in a syringe and then dispensed into a Ca-containing bath (150 mM $CaCl_2$) in order to rapidly solidify the deposit, as schematically illustrated in Fig. 1. The dispensation pressure was adjusted to 500 kPa using a regulator (IEI, AD2000C). The size was controlled by means of different needle gauges (23–27 G). After dispensing the materials into 10 ml of Ca-containing solution within a cylindrical mold (φ = 10 mm) the fibrous deposits were further pressed down manually to produce a disc-shaped scaffold of specific height. The process of depositing scaffolds within the Ca-containing bath took about 1 min. The height of the scaffold was varied in order to obtain different levels of porosity (1.2 mm for low, 1.5 mm for medium and 2.0 mm for high porosity). Likewise, the amount (weight) of scaffold material to be dispensed was varied (0.5 g for low, 0.4 g for medium and 0.3 g for high porosity) while the height of the scaffolds was kept constant (3 mm) to give different levels of porosity. The as-hardened scaffolds were used for further in vitro cell assays and in vivo animal studies without further treatment, such as soaking in water.

2.3. Characterization of 3-D porous scaffolds

The composite scaffolds obtained were thoroughly washed in distilled water and then immersed in simulated body fluid (SBF containing 142.0 mM Na^+, 5 mM K^+, 1.5 mM Mg^{2+}, 2.5 mM Ca^{2+}, 147.8 mM Cl^-, 4.2 mM HCO_3^-, 1.0 mM HPO_4^{2-}, 0.5 mM SO_4^{2-}) at 37 °C for periods of up to 7 days. Samples were washed and dried under vacuum and the morphology was examined by scanning electron microscopy (SEM) (Hitachi S-3000H). Composition change was monitored by energy dispersive spectroscopy (EDS) (Bruker SNE-3000 M) in a scanning electron microscope. An X-ray diffractometer (Rigaku Ultima IV) was used to detect changes in the crystalline phases of the scaffolds. The pore structure of scaffolds with different porosities was analyzed by micro-computed tomography (μCT) (Skyscan model 1172). A disc (φ 10 × 3 mm) of each sample was placed with the top and bottom surfaces parallel to the scanning plane. Scanning was performed with a 11 Mp X-ray camera and 758 files were acquired with an image pixel size of 19.92 μm. The surface charge of the α-TCP particles was determined by measurement of the zeta potential (Zetasizer ZEN3600, Malvern Instruments). The α-TCP particles were sieved (45 μm) and dispersed in distilled water at 1 mg ml^{-1} and the zeta potential was measured at room temperature and pH 7.0 using a disposable capillary cell (DTS1060C) and Zetasizer software (v. 6.20). The measurement was repeated on three different samples.

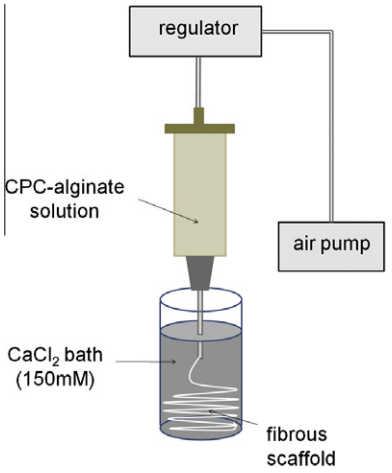

Fig. 1. Schematic drawing of the processing set-up to prepare calcium phosphate cement (CPC)/alginate composite (CPA) fibrous network for use as a 3-D tissue engineering scaffold. CPC–alginate suspension was injected through a needle using an air pump regulator into a cylindrical mold containing $CaCl_2$ (150 mM) which promotes rapid setting of the composite suspension.

The elastic modulus of the scaffolds was measured by means of dynamic mechanical analysis (DMA) (DMA25, Metravib, France). Samples with three different porosities were prepared with the dimensions 5 mm diameter × 10 mm height to which a dynamic compression load was applied. The storage modulus of the samples was recorded. Three samples were tested for each group.

2.4. Assas of protein delivery capacity

Protein release from the CPC–alginate porous scaffold was assessed using bovine serum albumin (BSA) and lysozyme as the model proteins. Loading of each protein was carried out in two different ways: one was to add the protein to the alginate solution and then mix this with CPC powder, which was subsequently deposited in a protein-containing porous scaffold (“loading I”); the other was to add the protein to the CPC suspension, which was incubated for 1 h with gentle agitation, and then the solution was mixed with alginate solution which was then deposited in a porous scaffold (“loading II”). Protein content in each scaffold sample was set at 33.3 μg mg $scaffold^{-1}$. 1 g of the protein-containing porous scaffold was used for the protein release test. This was