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Fabrication of macroporous cement scaffolds using PEG particles: In vitro evaluation with induced pluripotent stem cell-derived mesenchymal progenitors

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ABSTRACT

Calcium phosphate cements (CPCs) have been extensively used in reconstructive dentistry and orthopedics, but it is only recently that CPCs have been combined with stem cells to engineer biological substitutes with enhanced healing potential. In the present study, macroporous CPC scaffolds with defined composition were fabricated using an easily reproduced synthesis method, with minimal fabrication and processing steps. Scaffold pore size and porosity, essential for cell infiltration and tissue ingrowth, were tuned by varying the content and size of polyethylene glycol (PEG) particles, resulting in 9 groups with different architectural features. The scaffolds were characterized for chemical composition, porosity and mechanical properties, then tested in vitro with human mesenchymal progenitors derived from induced pluripotent stem cells (iPSC-MPs). Biomimetic decellularized bone scaffolds were used as reference material in this study. Our manufacturing process resulted in the formation of macroporous monetite scaffolds with no residual traces of PEG. The size and content of PEG particles was found to affect scaffold porosity, and thus mechanical properties. Irrespective of pore size and porosity, the CPC scaffolds fabricated in this study supported adhesion and viability of human iPSC-MPs similarly to decellularized bone scaffolds. However, the architectural features of the scaffolds were found to affect the expression of bone specific genes, suggesting that specific scaffold groups could be more suitable to direct human iPSC-MPs in vitro toward an osteoblastic phenotype. Our simplistic fabrication method allows rapid, inexpensive and reproducible construction of macroporous CPC scaffolds with tunable architecture for potential use in dental and orthopedic applications.

1. Introduction

The need for bone tissue substitutes for dental, craniofacial, and orthopedic reconstructions is rapidly increasing due to global population growth and extension of life expectancy [1,2], with the number of elderly people (+65 years) estimated to be about 2 billion by 2050 [3,4]. The available options to treat bone deficiencies are based on transplantation of bone grafts or implantation of alloplastic materials [5,6]. These treatments can restore tissue integrity and functionality but fail to provide optimal therapeutic solutions in several clinical cases, such as in situations characterized by extensive tissue loss, poor bone quality, or otherwise compromised regenerative capacity [7]. In contrast, engineering bone with osteocompetent cells and compliant biomaterials allows for growing an unlimited amount of tissue grafts with enhanced regenerative properties and broader clinical use [8].

Ceramics are among the most studied synthetic biomaterials in reconstructive orthopedics, and calcium phosphate cements (CPC) represent more than half of all ceramics under investigations. Many CPC formulations are FDA approved and have been used in clinical settings for a variety of applications, including cranioplasty, bone augmentation, implant fixation, fracture, and vertebroplasty [9,10]. CPC materials display similar chemistry as the mineral phase of bone, can be fabricated into different shape at a low cost, and are biocompatible and osteotransductive (they are replaced by bone tissue after implantation in human patients) [11,12].

Researchers have recently combined CPC scaffolds with cells to grow biological substitutes with enhanced regeneration potential [13–29]. However, only few of these studies have used macroporous cement scaffolds to sustain tissue formation in vitro [18,22–25], and little is known on the effect of porosity and pore size of macroporous cement scaffolds, monetite in particular, on cell behavior.

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In the present study, we report a simple synthesis method for fabrication of macroporous CPC scaffolds with defined composition and variable porosity. The scaffolds were fabricated by mixing monocalcium phosphate monohydrate and beta tri-calcium phosphate with PEG particles. Pore size and porosity were tuned by varying the size and content of PEG particles, resulting in 9 groups with different architectural features. Following fabrication, the scaffolds were characterized for morphology, chemical composition, porosity and mechanical properties, then examined in vitro with human mesenchymal progenitors to assess their potential to support cell growth and expression of bone specific genes.

2. Materials and methods

2.1. Scaffold fabrication

Polyethylene glycol (PEG; 20,000 MW, Sigma, St. Louis MO) was melted at 100°C before solidifying at room temperature and ground to a fine powder. The milled powder was sieved to defined size ranges of 100–400, 400–600 or 100–600 μm. PEG particles were added directly to the cement powder at a weight ratio of 0.4, 0.6, 0.8 or 1 g of PEG powder per gram of cement (subsequently referred to as 0.4, 0.6, 0.8, and 1 g/g), and mixed together in a turbula for 30 min. The cement powder was composed of mono-calcium phosphate monohydrate (MCPM, Scharlaeu, Spain) and beta tri-calcium phosphate (β-TCP, Sigma) at a 55 to 45 molar ratio [30]. The MCPM powder was sieved to include only 25–50 and 50–75 μm particles. Crystal growth inhibitors disodium dihydrogen pyrophosphate (1% by weight) and citric acid (2.4% by weight) were added directly to the powder and liquid.

The macroporous cements were fabricated by addition of the liquid, containing citric acid (0.5 M), to powder at a ratio of 0.25 ml per gram of cement (irrespective of the content of PEG). The mixture was shaken in a cap mixer for 1 min and immediately manually transferred to silicone molds (8 mm diameter × 3 mm thick) using a spatula. Within 5–7 min samples were transferred to calcium free Dulbecco's phosphate buffered saline (DPBS, Sigma) to allow for complete setting at 37°C for 24 h. After 24 h samples were removed from the molds, polished with sand paper to a thickness of about 2 mm, and transferred to fresh DPBS (calcium free) at 70°C to melt and leach the PEG for 48 h. The absence of PEG was confirmed by differential scanning calorimetry (DSC) and X-ray diffraction analysis (XRD) as described below. Each sample was dried at 70°C for 16 h, then sterilized in an autoclave at 150°C for 30 min and used for characterization and cell culture. Ten scaffold groups were thus manufactured as listed in Table 1.

Decellularized bone scaffolds (reference material) were prepared as previously described [31]. Plugs of trabecular bone (8 mm diameter) were drilled from the subchondral region of meta-carpal joints of calves (Green Village Packing, Green Village, NJ). Soon after, plugs were cleansed under high-pressure streamed water to remove the bone marrow and then sequentially washed with a solution of ethylenediamine tetra-acetic acid (EDTA) (0.1%) in DPBS, EDTA (0.1%) in Tris (10 mM), and SDS (0.5%) in Tris (10 mM), followed by treatment with a solution of DNase and RNase in Tris buffer (10 mM) to remove cellular material. Decellularized bone plugs were thoroughly rinsed in DPBS, freeze-dried, cut and then polished to a thickness of about 2 mm. Each individual scaffold was weighed and measured to calculate the density, and those in the range of 0.27–0.35 mg/mm^3 were selected and used for material characterization analysis and cell culture. For cell culture, scaffolds were sterilized overnight in ethanol (70% by volume) and then conditioned in expansion medium overnight.

Table 1
List of cement scaffolds used in this study.

Group	PEG content (g/g of cement)	PEG size (μm)
Ctrl	0	na
1	0.4	100–400
2	0.4	100–600
3	0.6	100–400
4	0.6	100–600
5	0.6	400–600
6	0.8	100–400
7	0.8	100–600
8	0.8	400–600
9	1	100–600

2.2. SEM analysis

Cement and decellularized bone scaffolds were imaged on a Hitachi TM100 scanning electron microscope (Hitachi High Technologies America Inc., Schaumburg, IL). Three random fields, from three samples per group, were photographed using the following settings: 15 kV, 100× magnification, 7 mm working distance.

2.3. Material composition analysis

X-ray diffraction and Rietveld analysis were used to assess the material composition after the manufacturing process. Briefly, the ground powder from autoclaved cement scaffolds was analyzed with XRD using a C8 advanced Bruker (Bruker Corporation, Billerica, MA), theta-theta setup at 40 kV and 40 mA and over diffraction angles of 5–60°. The step length was 0.3 s per step, with 0.01° per step and a rotation speed of 80 rotations per minute (rpm). Open source software Profex (http://profex.doebelin.org) was used to quantify the XRD spectra via Rietveld refinement. The reported values are the average of 3 readings for each group. Complete removal of PEG from leached scaffolds were confirmed by the lack of PEG peaks at angles 19.23° and 23.24° for samples for each group.

2.4. Differential scanning calorimetry (DSC)

Complete removal of PEG was also confirmed by DSC. Cylinders 8 mm in diameter and 3 mm in thickness were prepared for cell culture and during the leaching process samples were removed after 2.5, 24, 32 or 56 h of leaching, and dried for 2 h at 25°C. Three samples from each group were ground to a fine powder and 10–20 mg of powder was heated from 25°C to 120°C, at 10°C per minute, in a TA Q2000 DSC (TA Instruments, New Castle, DE). The total enthalpy change for each sample was calculated by TA universal analysis software (TA Instruments) and compared to cement powders containing a known content of PEG, and data were exported to graphing software Origin (OriginLab Corporation, Northampton, MA).

2.5. Porosity measurement

The porosity of cement and decellularized bone scaffolds was determined with the Acupyc 1330 helium pycnometry (Micrometrics, Norcross, GA). Six samples were ground to a fine powder with mortar and pestle and the density of the powder (skeletal density, ρ_s) determined by helium gas intrusion, in a chamber size of 1 cm^3, with 10 runs at 19.5 pound per square inch of pressure. Samples were first dried completely and the apparent density (ρ_a) was determined by measuring the physical dimensions of each specimen with calipers. The total porosity ($\Phi\%$) of samples was determined according to the following equation [32]:

$$\Phi\% = \left(1 - \frac{\rho_a}{\rho_s}\right) * 100 \quad (1)$$

The macroporosity was calculated for each group by substituting the average apparent density of the control group (no PEG) composites for the skeletal density in Eq. (1).