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Mineralized gelatin methacrylate-based matrices induce osteogenic differentiation of human induced pluripotent stem cells

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ABSTRACT

Human induced pluripotent stem cells (hiPSC) are a promising cell source with pluripotency and self-renewal properties. Design of simple and robust biomaterials with an innate ability to induce lineage-specificity of hiPSC is desirable to realize their application in regenerative medicine. In this study, the potential of biomaterials containing calcium phosphate minerals to induce osteogenic differentiation of hiPSC was investigated. hiPSC cultured using mineralized gelatin methacrylate-based matrices underwent osteogenic differentiation ex vivo, in both two-dimensional and three-dimensional cultures, in growth medium devoid of any osteogenic-inducing chemical components or growth factors. The findings that osteogenic differentiation of hiPSC can be achieved through biomaterial-based cues alone present new avenues for personalized regenerative medicine. Such biomaterials that could not only act as structural scaffolds, but could also provide tissue-specific functions such as directing stem cell differentiation commitment, have great potential in bone tissue engineering.

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1. Introduction

Human pluripotent stem cells (hPSC), which include both embryonic stem cells and induced pluripotent stem cells, play an important role in regenerative medicine, developmental biology and pathology, and drug screening, owing to their ability to give rise to any cells in the human body and indefinitely self-renew [1,2]. hiPSC developed from human autologous somatic cells could circumvent concerns regarding immune properties and ethical issues, making them an ideal cell source for regenerative medicine [3]. Despite the benefits that hiPSC offer, controlling their differentiation into targeted cell type(s) remains a challenge. Studies over the years have shown that stem cells respond to their microenvironment, composed of soluble and matrix-based cues, to regulate their fate and commitment [4–6]. Synthetic biomaterials have been used extensively to recapitulate tissue-specific physicochemical cues to direct self-renewal and differentiation of stem cells [7,8].

Biomaterials containing calcium phosphate minerals have been shown to promote osteogenic differentiation of stem cells [9–14]. These materials have also been shown to support in vivo bone tissue formation [11–15]. Previously, it has been shown that hydrogels containing acryloyl-6-aminocaproic acid (A6ACA) moieties promote their mineralization when exposed to a medium containing Ca^{2+} and PO_4^{3-} [16]. The carboxyl groups of A6ACA moieties bind to Ca^{2+} ions and promote nucleation and growth of calcium phosphate (CaP) minerals. Employing biomineralized poly(ethylene glycol)-diacrylate-*co*-acryloyl-6-aminocaproic acid (PEGDA-*co*-A6ACA) matrices, it has been shown that mineralized matrices can direct osteogenic differentiation of human bone marrow-derived mesenchymal stem cells (hMSC) and human embryonic stem cells (hESC) [10,17]. However, it required the biomineralized PEGDA-*co*-A6ACA matrices to be coated with Matrigel to promote initial attachment of the hESC to the matrix. In this study, mineralized matrices containing gelatin methacrylate (GelMA) were developed, and their potential to direct osteogenic differentiation of hiPSC was examined. Gelatin, derived from natural collagen, possesses cell adhesion motifs that could promote adhesion of hiPSC to the underlying matrix [18,19]. Moreover, gelatin-based matrices have been demonstrated to degrade [19–24] and have been studied extensively as a scaffold for tissue engineering [20,21,23,25].

Studies that have reported osteogenic differentiation of hiPSC often used derivation of MSC or mesoderm-like progenitor cells

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and their subsequent differentiation into osteoblasts, using osteogenic-inducing soluble factors such as β-glycerophosphate, ascorbic acid 2-phosphate, dexamethasone and/or growth factors such as bone morphogenetic protein-2 (BMP-2) [26–32]. A recent study by de Peppo et al. [27] employed decellularized bone matrix to create bone tissues from hiPSC-derived mesoderm progenitor cells. However, to the present authors' knowledge, there are no reports showing osteogenic differentiation of hiPSC solely through biomaterial-based cues. The present study demonstrates that biomaterials containing CaP minerals induce osteogenic differentiation of hiPSC in growth medium devoid of any osteogenic-inducing small molecules or growth factors.

2. Materials and methods

2.1. Synthesis and modification of materials

PEGDA (M_n = 3.4 kDa) and *N*-acryloyl 6-aminocaproic acid (A6ACA) were synthesized as previously described [17,33,34]. GelMA was prepared through methacrylation of gelatin (Sigma-Aldrich, catalog number: G1890) [19]. Briefly, 10 g of gelatin along with 100 ml of phosphate buffered saline (PBS) was added to a round-bottom flask purged with argon gas and dissolved under stirring at 60 °C. Around 8 ml of methacrylic anhydride (Polysciences, catalog number: 01517) was added dropwise under stirring for 2 h. The reaction mixture was kept at 60 °C for another 1 h, then 100 ml of PBS pre-warmed at 60 °C was added to the mixture and maintained at 60 °C for 15 min. The resulting GelMA was placed in a dialysis tube (Spectrum Laboratories, catalog number: 132676) in deionized (DI) water at 40 °C for 7 days with two daily changes of DI water and filtered through 40-μm-sized pores, lyophilized and stored at −20 °C prior to use.

2.2. Synthesis of GelMA-co-A6ACA hydrogels

GelMA-*co*-A6ACA hydrogels were synthesized as follows: 30 w/v% GelMA was dissolved in DI water at 45 °C, and 1 M A6ACA was dissolved in 1 M NaOH to deprotonate the carboxyl groups. One part each of 30 w/v% GelMA and 1 M A6ACA were mixed to yield a solution containing 15 w/v% GelMA and 0.5 M (equivalent to 9 w/v%) A6ACA. Around 0.3 w/v% of photoinitiator, 1-[4-(2-hydroxyethoxy)-phenyl]-2-hydroxy-2-methyl-1-propane-1 (Ciba Specialty Chemicals, Irgacure 2959), in 70% ethanol was added to the above solution. The solution was then dispensed into a Bio-Rad glass plate separated by a 1-mm spacer and allowed to polymerize at 25 °C under 365 nm UV light for 10 min. The resultant hydrogels were incubated in PBS for 24 h with two changes of PBS. Hydrogels disks 1 cm^2 (area) × 1 mm (height) were used for the cell culture experiments.

2.3. Synthesis of GelMA-co-A6ACA-co-PEGDA macroporous hydrogels

GelMA-*co*-A6ACA-*co*-PEGDA macroporous hydrogels were synthesized using the polymethylmethacrylate (PMMA) leaching method [35]. First, 8-mm-diameter cylindrical molds packed with PMMA were made from PMMA microspheres 165 μm in diameter (Bangs Laboratories, catalog number: BB05 N). Each PMMA column was exposed to 60 μl of 20% acetone/80% ethanol mixture for 1 min to fuse the PMMA beads. The mold was dried at 80 °C for 1 h and stored at room temperature. Next, 50 μl of a precursor solution containing 10 w/v% GelMA, 9 w/v% A6ACA (treated with NaOH), 10 w/v% PEGDA, and 0.3 w/v% Irgacure 2959 (in 70% ethanol) was dispensed into PMMA-filled molds and photopolymerized for 10 min using UV light. The PMMA beads embedded within the GelMA-*co*-A6ACA-*co*-PEGDA networks were subsequently dissolved in acetone for 3 days, while replenishing fresh acetone three times each day, yielding the macroporous hydrogels. The resultant macroporous hydrogels were gradually hydrated from pure acetone to acetone/DI water mixture and to pure DI water for a day. The macroporous hydrogels were equilibrated in PBS for 6 h and punched out to obtain constructs with diameter and height dimensions of 5 mm and 2 mm, respectively.

2.4. Mineralization and sterilization of GelMA-co-A6ACA hydrogels and GelMA-co-A6ACA-co-PEGDA macroporous hydrogels

GelMA-*co*-A6ACA hydrogels and GelMA-*co*-A6ACA-*co*-PEGDA macroporous hydrogels were subjected to the mineralization process, as described elsewhere [17]. Briefly, both matrices were soaked in DI water for 6 h and immersed in modified simulated body fluid (m-SBF; pH = 7.4) at 25 °C for 6 h. The m-SBF solution is composed of 142.0 mM Na^+, 5.0 mM K^+, 1.5 mM Mg^{2+}, 2.5 mM Ca^{2+}, 103.0 mM Cl^-, 10.0 mM HCO_3^-, 1.0 mM HPO_4^{2-} and 0.5 mM SO_4^{2-}, as described elsewhere [36]. The matrices were briefly rinsed with DI water and soaked in 40 mM Ca^{2+} and 24 mM HPO_4^{2-} solution (pH = 5.2) at 25 °C for 45 min using a VWR Mini Shaker (catalog number: 12620-938) at 200 rpm. The matrices were then briefly rinsed in DI water, incubated in m-SBF at 37 °C for 2 days with daily change of m-SBF, and equilibrated in PBS for 6 h.

The mineralized and non-mineralized matrices were sterilized by immersing in 70% ethanol for 6 h. The ethanol-treated matrices were then washed in sterile PBS by replenishing the PBS four times each day for 4 days to fully remove residual ethanol. Sterile non-mineralized and mineralized GelMA-*co*-A6ACA hydrogels were employed for two-dimensional (2-D) culture. Sterile non-mineralized and mineralized GelMA-*co*-A6ACA-*co*-PEGDA macroporous hydrogels were used for three-dimensional (3-D) culture.

2.5. Scanning electron microscopy and energy dispersive spectra

Scanning electron microscopy (SEM) imaging was carried out to investigate the morphology of the mineralized matrices. Energy dispersive spectra (EDS) analysis was performed to determine the composition of the minerals. Samples were briefly rinsed in DI water to remove non-bound ions for 5 min, cut into thin slices and subjected to flash-freezing and lyophilization. After iridium coating for 7 s in the sputter (Emitech, K575X), samples were imaged using SEM (Philips XL30 ESEM) and analyzed for elemental spectra with an integrated EDS system. INCA software was used to quantify Ca/P atomic ratio from elemental spectra. The pore diameter of the macroporous matrices was calculated from either SEM or bright-field images to estimate the pore structures in the dry and wet state, respectively. Roughly 10 pores were chosen from each of three SEM or bright-field images (n = 30) and their diameter was determined using ImageJ. The data are presented as mean ± standard errors.

2.6. Calcium and phosphate assays

Calcium and phosphate assays were conducted to determine the amounts of Ca^{2+} and PO_4^{3-} in the mineralized matrices and to determine the dissolution of the minerals from the mineralized matrices. The matrices were rinsed in DI water, homogenized and freeze-dried, and their dry weights were measured. To measure the Ca^{2+} and PO_4^{3-} contents of the mineralized matrices, the dried matrices were subjected to vigorous shaking in 0.5 M HCl at 25 °C for 3 days. To examine the dissolution of CaP minerals from the mineralized matrices into Ca^{2+} and PO_4^{3-} in an environment lacking these ions, equilibrium swollen matrices were incubated in 1.5 ml of 50 mM Tris buffer (pH = 7.4), at 37 °C for 7 days, and 0.3 ml of incubation medium was collected and

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