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Comparative effect of physicomechanical and biomolecular cues on zone-specific chondrogenic differentiation of mesenchymal stem cells

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ABSTRACT

Current tissue engineering approaches to regeneration of articular cartilage rarely restore the tissue to its normal state because the generated tissue lacks the intricate zonal organization of the native cartilage. Zonal regeneration of articular cartilage is hampered by the lack of knowledge for the relation between physical, mechanical, and biomolecular cues and zone-specific chondrogenic differentiation of progenitor cells. This work investigated in 3D the effect of TGF-β1, zone-specific growth factors, optimum matrix stiffness, and adding nanofibers on the expression of chondrogenic markers specific to the superficial, middle, and calcified zones of articular cartilage by the differentiating human mesenchymal stem cells (hMSCs). Growth factors included BMP-7, IGF-1, and hydroxyapatite (HA) for the superficial, middle, and calcified zones, respectively; optimum matrix stiffness was 80 kPa, 2.1 MPa, and 320 MPa; and nanofibers were aligned horizontal, random, and perpendicular to the gel surface. hMSCs with zone-specific cell densities were encapsulated in engineered hydrogels and cultured with or without TGF-β1, zone-specific growth factor, optimum matrix modulus, and fiber addition and cultured in basic chondrogenic medium. The expression of encapsulated cells was measured by mRNA, protein, and biochemical analysis. Results indicated that zone-specific matrix stiffness had a dominating effect on chondrogenic differentiation of hMSCs to the superficial and calcified zone phenotypes. Addition of aligned nanofibers parallel to the direction of gel surface significantly enhanced expression of Col II in the superficial zone chondrogenic differentiation of hMSCs. Conversely, biomolecular factor IGF-1 in combination with TGF-β1 had a dominating effect on the middle zone chondrogenic differentiation of hMSCs. Results of this work could potentially lead to the development of multilayer grafts mimicking the zonal organization of articular cartilage.

1. Introduction

During articular cartilage development, mesenchymal stem cells (MSCs) condense in morphologically distinct zones that reflect the spatiotemporal gradient of chondrogenic signaling factors [1]. The differentiated cells form distinct zones, namely, superficial zone for lubrication, middle/deep zone for compressive strength and resisting deformation, and calcified zone for load transmission to the underlying bone tissue [2–4]. Each zone is maintained by a unique combination of cellular, biomolecular, mechanical, and physical factors. Osteoarthritis (OA) or degenerative joint disease affects 27 M Americans with joint pain and disability [5]. Current treatment methods such as autograft transfer or autologous chondrocyte transplantation rarely restore the tissue to its normal state [6]. There is a need for engineered grafts that recreate the zonal organization of articular cartilage for treating full-thickness articular cartilage defects such as those in advanced OA.

Zonal organization of articular cartilage during development is formed by controlling the secretion and spatial distribution of transforming growth factor-β (TGF-β), bone morphogenetic protein (BMP), and insulin growth factor (IGF) family of growth factors [7], which results in 450% increase in tensile and 180% increase in compressive modulus with depth from the articular surface [8]. The outermost superficial zone is maintained by TGF-β1 and BMP-7 signaling, a relatively low matrix stiffness (high compliance, 80 kPa), and collagen fibrils oriented parallel to the articulating surface [9]. Chondrocytes in the superficial zone express pre-chondrogenic marker Sox-9 and superficial zone protein (SZP) for

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joint lubrication [3,9]. The middle zone is maintained by TGF-β1 and IGF-1 signaling, a matrix stiffness much higher than the superficial zone (2.1 MPa), and collagen fibrils with random alignment [10,11]. Pre-hypertrophic chondrocytes in the middle zone highly express aggrecan (AGC), glycosaminoglycans (GAG), and collagen type II (Col II) [12,13]. Hypertrophic chondrocytes in the calcified zone are maintained by TGF-β1 signaling, high matrix stiffness (320 MPa), and collagen fibrils oriented perpendicular to the articulating surface [14]. Chondrocytes in the calcified zone express hypertrophic markers alkaline phosphatase (ALP) and collagen type X (Col X) [13].

In mature articular cartilage tissue, matrix stiffness increases sharply from 80 kPa in the superficial zone to 2.1 MPa in the middle zone and 320 MPa in the calcified zone [15,16]. Concurrent with increase in matrix stiffness, orientation of the collagen fibrils changes from parallel to the articulating surface in the superficial zone to random and perpendicular in the middle and calcified zones, respectively [13,17]. In addition to those gradients, the optimum TGF-β1 loading increases from 3 ng/mL in the superficial zone to 30 ng/mL in the middle and calcified zones [9,18]. Further, the expression of BMP-7 [9] and IGF-1 [19] is restricted to the superficial and middle zones, respectively, whereas the secretion of hydroxyapatite (HA) by hypertrophic chondrocytes further stimulates mineralization in the calcified zone [20].

We recently demonstrated using a developmentally inspired approach that the combination of TGF-β1 as a master regulator of chondrogenesis and zone-specific growth factor, matrix stiffness, and nanofiber orientation led to zone-specific chondrogenic differentiation of encapsulated human mesenchymal stem cells (hMSCs) and expression of corresponding zone-specific markers for the superficial, middle, and calcified zones [18]. The effects of biomolecular factors like TGF-β1, BMP-7, IGF-1, and HA, addition of nanofibers and their orientation, and mechanical stiffness on chondrogenic differentiation of MSCs and chondroprogenitor cells have been investigated extensively in the past [20–22]. However, independent effect of the above factors on differentiation of encapsulated MSCs to zone-specific chondrocyte phenotypes of articular cartilage has not been investigated. This work investigated for the first time the effect of TGF-β1, zone-specific growth factors (BMP-7 for superficial, IGF-1 for middle, and HA for calcified zone), optimum matrix compressive modulus (80 kPa for superficial, 2.1 MPa for middle, and 320 MPa for calcified zone), and nanofibers (horizontal for superficial, random for middle, and perpendicular to the articulating surface for calcified zone) on zone-specific chondrogenic differentiation of hMSCs. An engineered hydrogel based on star acrylate-terminated lactide-chain-extended polyethylene glycol macromer (SPELA) with tunable modulus and degradation time was used for hMSC encapsulation.

2. Materials & methods

2.1. Materials

Polyethylene glycol (PEG) with nominal molecular weight (MW) of 4.6 kDa was received from Acros Organics (Fairfield, OH). Poly(DL-lactide) (PLA) with MW of 95 kDa was received from Durect (Birmingham, AL). Photoinitiator 4-(2-hydroxyethoxy) phenyl-(2-hydroxy-2-propyl) ketone (Irgacure 2959) was received from CIBA (Tarrytown, NY). Hydroxyapatite (HA) nanoparticles with long axis of 80 nm and aspect ratio of 4 were received from Berkeley Advanced Biomaterials (Berkeley, CA) [23]. All Fmoc-protected amino acids, the Rink Amide NovaGel™ resin and hydroxybenzotriazole (HOBt) were purchased from EMD Biosciences (San Diego, CA). 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), N,N-dimethylformamide (DMF), dichloromethane (DCM), 4-dimethylaminopyridine (DMAP), diisopropylcarbodiimide (DIC), triisopropylsilane (TIPS), trifluoroacetic acid (TFA), hexane and diethyl ether were received from Acros. Calcium hydride, 4,6-diamidino-2-phenylindole (DAPI), paraformaldehyde, triethylamine (TEA), penicillin G, insulin, papain, dithiothreitol, EDTA and streptomycin were received from Sigma-Aldrich (St. Louis, MO). Spectro/Por dialysis tube (MW cutoff 3.5 kDa) was from Spectrum Laboratories (Rancho Dominquez, CA). DCM solvent was purified by distillation over calcium hydride. All other solvents were reagent grade and used as received. Human mesenchymal stem cells (hMSCs) with high expression of CD105, CD166, CD29, and CD44 and low expression of CD14, CD34 and CD45 markers, were received from Lonza (Allendale, NJ). According to the supplier, the bone marrow was harvested from the posterior iliac crest of one donor (normal, Caucasian, male, 38 years old). Human chondrocytes with 90% positive staining for Alcian blue were received from Lonza. According to the supplier, chondrocytes were isolated from the hyaline cartilage in the knee of one donor (normal, Caucasian, male, 50 years old). Transforming growth factor-β1 (TGF-β1) and Insulin Growth Factor-1 (IGF-1) were purchased from Lonza (Allendale, NJ). Bone morphogenetic protein-7 (BMP-7) and bovine serum albumin (BSA) were received from Novus (Littleton, CO) and Jackson ImmunoResearch (West Grove, PA), respectively. Iodoacetic acid was purchased from Fisher Scientific (Rockford, IL). DMEM cell culture medium, Dulbecco's phosphate-buffer saline (PBS), fetal bovine serum (FBS), trypsin-EDTA, Quant-iT Pico-Green dsDNA reagent kit and Alexa Fluor 594 Phalloidin were received from Invitrogen (Carlsbad, CA). All primary and secondary antibodies, luminol reagent and Blotto blocking solution were received from Santa Cruz Biotechnology (Dallas, TX). All forward and reverse primers were synthesized by Integrated DNA Technologies (Coralville, IA).

2.2. Synthesis of fiber reinforced hydrogels

SPELA macromonomer was synthesized by ring-opening chain extension of PEG with a short lactide at each chain-end followed by termination with an acrylate group as we described previously [24,25] (see Fig. 1 for structure). Acrylamide-terminated glycine-arginine-glycine-aspartic acid peptide (Ac-GRGD) was synthesized on Rink Amide NovaGel™ resin in the solid phase as we previously described [26]. Random and aligned PLA nanofiber microsheets were generated by electrospinning from 10 wt% solution of PLA in HFIP as we previously described [27]. The hydrogel precursor solution was prepared by mixing initiator solution (5 mg initiator in 1 mL PBS) with the solution of SPELA macromonomer and Ac-GRGD cell-adhesive peptide in PBS. The hydrogel precursor solution (with or without cells and nanofibers) was crosslinked by ultraviolet (UV) polymerization with an Omni Cure Series 1500 UV illumination system for 30 S as we previously described [18]. SPELA10, SPELA7.5 and SPELA5 gels with lactide to PEG molar feed ratio of 10, 7.5 and 5, respectively, with mass loss of 47%, 38%, and 28% after 21 days in PBS at 37 °C for the gels simulating the superficial, middle, and calcified zones of articular cartilage, respectively [28] (Table 1). Compressive moduli of the superficial, middle and calcified zones were simulated with the following fiber-reinforced hydrogels: 15 wt% SPELA10 and 4 wt% (by the gel weight) PLA nanofibers oriented parallel with respect to surface of the disk-shape gel for the superficial zone; 50 wt% SPELA7.5 with 4 wt% randomly-oriented fibers for the middle zone; and 35 wt% SPELA5 with 60 wt% perpendicularly-oriented fibers for the calcified zone (Fig. 1). Addition of nanofibers to the superficial and middle zones did not significantly change their modulus in perpendicular direction with respect to the gel surface whereas SPELA5 gel had to be reinforced with nanofibers to reach the required 320 MPa modulus