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Hydrophilic polyurethane matrix promotes chondrogenesis of mesenchymal stem cells ☆

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ABSTRACT

Segmental polyurethanes exhibit biphasic morphology and can control cell fate by providing distinct matrix guided signals to increase the chondrogenic potential of mesenchymal stem cells (MSCs). Polyethylene glycol (PEG) based hydrophilic polyurethanes can deliver differential signals to MSCs through their matrix phases where hard segments are cell-interactive domains and PEG based soft segments are minimally interactive with cells. These coordinated communications can modulate cell–matrix interactions to control cell shape and size for chondrogenesis. Biphasic character and hydrophilicity of polyurethanes with gel like architecture provide a synthetic matrix conducive for chondrogenesis of MSCs, as evidenced by deposition of cartilage-associated extracellular matrix. Compared to monophasic hydrogels, presence of cell interactive domains in hydrophilic polyurethanes gels can balance cell–cell and cell–matrix interactions. These results demonstrate the correlation between lineage commitment and the changes in cell shape, cell–matrix interaction, and cell–cell adhesion during chondrogenic differentiation which is regulated by polyurethane phase morphology, and thus, represent hydrophilic polyurethanes as promising synthetic matrices for cartilage regeneration.

1. Introduction

Matrix guided cell-instructive cues are crucial for biomaterial based tissue regenerative applications. Synthetic materials should mimic the hierarchical architecture of native extracellular matrix to provide these cues to cells. In particular, biomaterial based strategies for cartilage regeneration are focused on developing provisional synthetic replacements which have structure and function resembling the native cartilage matrix and can support the cells in organizing into functional tissues. Native cartilage matrix is highly hydrated with 60–80% water content and the solid content is primarily biphasic with type II collagen and proteoglycans in a mesh-like network structure [1,2]. Chondrocytes derived from mesenchymal stem cells (MSCs) are resident cells of cartilage and are crucial for maintaining the extracellular matrix. These features contribute to the biophysical and mechanical properties of the cartilage matrix. Since cartilage exhibits limited intrinsic healing capacity, synthetic biomaterials are used to deliver therapeutically relevant cells including MSCs. Owing to the gel like architecture of native cartilage matrix, biomaterial based approaches for cartilage regeneration are focused on synthetic, semi-synthetic, and natural materials which can form gels with a high water content. Most widely used synthetic hydrogels are based on polyethylene glycol (PEG) while natural hydrogels are based on hyaluronic acid and alginate [3–7]. Gel-based materials are also preferable due to their ability to maintain the rounded morphology of cells, often considered a major factor for chondrogenesis of MSCs [8,9]. However, most of these gels do not mimic the biphasic structure of cartilage matrix. In addition these gels require chemical crosslinking to confine the cells within the matrix in a three-dimensional environment.

To address these features, we envisioned that segmental polyurethanes (PUs) with hydrophilic character can present a biphasic structure and form a gel-like architecture to produce a compatible synthetic matrix for chondrogenic differentiation of MSCs. PUs are essentially biphasic with soft and hard segments and segmental interactions. It is primarily H-bonding and electrostatic interactions drive the assembly of PU segments into a nanophase morphology [10–12]. Recently, our studies have shown that PU nanophases provide matrix-guided signals to control cell-fate by stimulating specific interactions [13]. However, these

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polyurethanes were unable to form gels with high water content due to their hydrophobic character. Therefore, in this study we aimed to use PEG based PUs which can form gel-like structures due to their high water absorption. Owing to the biphasic morphology of PUs and the ability to form hydrogels, PEG-PUs may mimic the functional characteristics of native cartilage matrix. Furthermore, PU gels do not require chemical crosslinking for stability as hard segments can assemble into distinct nanophasic structures due to non-covalent molecular interactions and, thus, allow hard segment domains to act as physical crosslinks between the soft segments. To assess, the ability of PEG based PUs to control MSCs toward chondrogenic lineage, we utilized PUs with PEG as the soft segment with aliphatic diisocyanates and L-tyrosine-based dipeptide chain extender as hard segment [14]. By altering PU structure, we investigated the role of biphasic PU structure in controlling MSCs for chondrogenic differentiation of MSCs. These PUs are also degradable and, therefore, can act as a temporary synthetic analog for cartilage regeneration [15]. Thus far, use of PUs for cartilage regeneration has been focused on porous scaffolds which cannot mimic the highly hydrated gel-like structure of the native cartilage matrix [16]. In this context, this approach represents advancement toward utilizing PUs as gels for chondrogenesis of stem cells.

2. Materials and methods

2.1. Materials

Polyethylene glycol (PEG) with number average molecular weight of 1000 was purchased from Sigma Aldrich and used after vacuum drying at 50 °C for 3 days to remove moisture. All other chemicals and solvents were purchased from Sigma Aldrich (MO) and were used as received unless otherwise noted. Desaminotyrosine tyrosyl hexyl ester (DTH) was synthesized according to a literature procedure [17]. Coverslips were purchased from Fisher Scientific (PA). Bone marrow derived MSCs were purchased from Texas A&M Health Science Center College of Medicine (supported through a grant from NCRR of the NIH). Cell culture medium (α-MEM), Alamar blue and 4,6-diamidino-2-phenylindole (DAPI) were purchased from Invitrogen, CA. Fetal bovine serum (FBS) was purchased from Atlanta Biologicals, GA, F-actin and focal adhesion staining kit (FAK100) from Millipore, MA, DNA quantification kit 'Quan-iT Picogreen' kit from Invitrogen, mouse anti-aggrecan from Santacruz Biotechnology, mouse anti-cadherin-11 from R&D systems and fluorescently labeled FITC and TRITC conjugated secondary antibodies from Millipore, MA were used. Collagen I and collagen II immunohistological staining was performed with commercial staining kits from Chondrex, Inc., WA and GAG and collagen II quantification was performed with quantification kits from Astrate Biologics, VA.

2.2. Polymer synthesis and substrate preparation

Polyurethanes were synthesized by a two-step process as described in prior publications [14]. Briefly, polyethylene glycol (PEG) and hexamethylene diisocyanate (HDI) or 4,4′-methylenebis(cyclohexyl isocyanate) (HMDI) (1:2 molar ratio) were reacted at 100 °C in dry DMF for 3 h in the presence of tin-2-ethyl hexanoate (0.1 mol%) as catalyst to form the prepolymer. After 3 h the reaction mixture was cooled to room temperature and DTH was added to it (PEG to DTH molar ratio was 1:1). The reaction mixture was stirred at 80 °C overnight. After cooling the reaction mixture to room temperature, it was poured into saturated sodium chloride solution and cooled in an ice bath to precipitate the polymer. Precipitated polyurethanes were filtered, washed with distilled water, and dried under vacuum at room temperature for 12 h. To form PU gels, precipitated polyurethane in aqueous media was aggregated by centrifugation to form a gel with entrapped water.

1 wt.% solutions of polyurethanes were prepared by dissolving the polymer in chloroform. Circular glass slides (18 mm diameter, .17 mm thickness) were coated by dipping the glass slide in the polymer solution and immediately drying it at room temperature. Poly(ethylene glycol) (PEG) substrates were prepared by photopolymerizing PEG diacrylate (MW: 1000) on coverslips using photoinitiator with UV light. Polyurethane coated cover slips and PEG substrates were transferred to a 12-well plate, washed twice with Phosphate Buffered Saline (PBS) followed by washing twice with cell culture medium (CCM).

2.3. Polyurethane phase morphology

Phase morphology of PU substrates was analyzed using atomic force microscopy (AFM), transmission electron microscopy (TEM), Fourier-transform infra-red spectroscopy (FT-IR), and wide angle X-ray diffraction (WAXD). AFM imaging of the polymer coated glass slides was done using the dynamic force mode of a Park Systems XE-100 AFM. A silicon cantilever with a nominal spring constant of 40 N/m, resonant frequency of 300 kHz and tip radius of 10 nm was used. The phase shift angle (phase difference between the piezo driver signal and the oscillation of the cantilever as detected by the photodetector) of the dynamic force mode AFM is sensitive to tip–sample interaction. A smaller phase shift angle (i.e., darker contrast in the phase image) suggests a soft segment and a larger phase shift angle (brighter contrast) suggests a hard segment. For TEM, the samples were prepared by drop-casting a 1% (w/v) solution of the polymer in chloroform on a carbon coated copper grid (Tedpella, 400 mesh size) followed by evaporation of the solvent at room temperature. The samples were stained with a 2% solution of phosphotungstic acid before imaging. TEM images were obtained using a Jeol JEM-2010 TEM working at an operating voltage of 200 keV. FTIR spectra were recorded using a Bruker Vortex 70 spectrometer in the wavenumber range of 4000–400 cm^{-1}. The spectral resolution was 4 cm^{-1} and 128 scans were averaged. Absorbance ratio was calculated from the intensity of respective FTIR peaks. WAXD was done using Rigaku Ultima IV X-ray diffractometer and scanning was done from 5 to 60° at a rate of 0.5° per minute.

2.4. MSC morphology on PU substrate

PU coated coverslips (and control PEG substrates) were placed in a 12-well plate and approximately 3500 MSCs were added in each well for a relatively low cell density to avoid cell–cell contact. This allowed for assessing cell–matrix interactions without significant interference from cell–cell interactions. Prior to cellular morphology analysis cell viability, adhesion, and proliferation were assessed as described in SI. Cell morphology was assessed from brightfield images of MSCs captured with Nikon Ti-U Inverted Microscope equipped with camera at 10×. For a given sample, multiple images were acquired from randomly selected fields and representative images are presented from each group. Circularity index (CI) of cells was computed as, $CI = 4\pi A / P^2$, where A is the area of the cell and P is the perimeter of cells. Thus, CI value 1 indicates perfect circle and a value close to zero indicates a non-circular thin shape. Cell area and perimeter were calculated from the brightfield image using NIS element software. For a given surface, approximately 20 cells were examined from randomly selected fields to determine the cell surface area and circularity index. The experiments were performed in triplicate for a given surface and the experiments were repeated three times. A representative result from a given experiment is presented. Structural organization of MSCs on different substrates was observed through staining of F-actin and focal adhesion protein vinculin using Actin Cytoskeleton/Focal Adhesion Staining Kit according to the manufacturers' protocol. Briefly, MSCs were fixed with 4% paraformaldehyde in PBS at room temperature for 15 min, washed twice with wash buffer (0.05% Tween-20 in PBS) and permeabilized with 0.1% Triton X-100 in PBS for 5 min at room temperature. Cells were washed twice with wash buffer followed by blocking with a 1% BSA solution in PBS for 30 min at room temperature. For focal adhesion staining, primary antibody (anti-vinculin) was diluted to a