Biophysical regulation of articular chondrocyte phenotype

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Statement of Purpose: The goal of this study was to test the topographical regulation of articular chondrocyte morphology in regulating phenotype.

Introduction: A major barrier limiting translation of *in vitro* osteoarthritis research into clinical disease-modifying therapies is chondrocytes rapidly de-differentiate under standard culture conditions.[1,2] A rounded morphology is critical to the maintenance of chondrocyte phenotype.[3] 3D culture platforms can promote a chondrogenic phenotype;[4-6] however, many of these maintain a rounded morphology by limiting adhesion, which is antithetical to *in vivo* conditions. Moreover, these techniques tend to have analytical restrictions, especially for observing sensitive post-translational protein modifications that are key to cell signaling pathways. To combat these limitations, we recently developed a micropatterned composite thin-film platform, the CellWell (Fig. 1A), to model articular cartilage *in vitro*.[7] The CellWell is designed with mechanically tuned hemi-spheroidal wells, precisely sized for individual cells. We previously reported that this design facilitates the promotion of a physiological rounded articular chondrocyte morphology for at least 28 days, 4x longer than similar previous techniques.[7] Our goal here was to assess the effects of that morphological maintenance on expression of phenotypic markers.

Methods: Primary human articular chondrocyte isolation from de-identified ankle articular cartilage was performed from N=3-7 donors per experiment as described previously.[7] Cells were plated on control substrates (2D agarose) or in CellWells with well diameters of 12, 15, and 18 µm that were functionalized with 2 mg/mL polydopamine and coated with 40 µg/mL fibronectin. Chondrocytes were maintained for up to 28 days at 37 °C in a 5% CO_2 environment under physiological (2%) O_2 conditions using a Biospherix (Parish, NY) subchamber system. Cells were fixed with 4% formaldehyde and permeabilized with 1% Tween-20 at 10 and 28 days after cell seeding, then immune-stained for markers of physiological phenotype. Positive markers of phenotype maintenance included type II collagen and aggrecan. Negative phenotype markers included types I and X collagen and Ki-67. Epifluorescence imaging was used to assess levels of protein expression of each of the phenotype markers. Images were manually segmented independently for each color channel using corresponding phase contrast images as a guide and analyzed on a single-cell basis.

Results: Cells remained viable for 28 days, with mean viability at day 28 of 78% ± 10% for cells on 2D agarose and 87% ± 3% for cells in CellWells. However, low cell densities were consistently observed (Fig. 1B), which we attribute to the noncovalent nature of using polydopamine for functionalization of agarose surfaces. We consistently observed that ~83% ±5% of the cells in CellWell substrates were located within wells, with 17% ± 5% located between wells at any given time point. Chondrocytes were found mostly within 12 µm (53% ±15%) and 15 µm (44% ±15%) diameter wells, with very few (2.8% ± 1.4%) found within 18 µm wells. We found that each of the phenotype markers investigated here was more appropriately expressed by chondrocytes in CellWell substrates than the mechanically and biochemically identical 2D agarose controls.

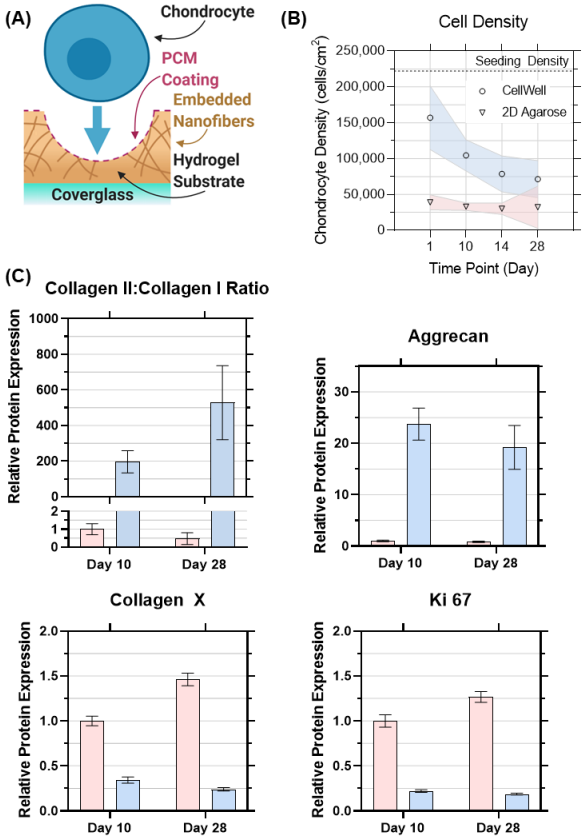

Figure 1: (A) Schematic of the CellWell. (B) Cell density was not well maintained on either substrate. (C) Phenotype markers were more physiological for chondrocytes in CellWells. Data are mean ± 95% CI.

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