INTRODUCTION: Chondrocytes occupy <10% of cartilage volume but are entirely responsible for the synthesis, maintenance, and turnover of the tissue's extracellular matrix. The pericellular matrix (PCM) regulates the biochemical signaling [1] as well as the mechanical microenvironment of the cell [2]. Fully developed PCM, rich in proteoglycans (PGs), hyaluronan, and type VI collagen [3], has been characterized mechanically via micropipette aspiration and shown to have a modulus (E = 66.5 kPa [4]) an order of magnitude higher than that of isolated chondrocytes with no PCM (E = 0.65 kPa [5]). Our objectives were to examine the influence of IGF-1 and OP-1 on the nanomechanical properties of individual chondrocytes and the extent and kinetics of development of their newly synthesized PCM. We used AFM nanoindentation with both nano and micron-sized probe tips to explore the relation between PCM biochemical composition and mechanical properties of the newly developing PCM.

METHODS: Cell culture: Chondrocytes were enzymatically isolated from immature bovine cartilage (1 hr treatment with 0.2% pronase and 16 hrs in 0.025% collagenase) and seeded in 2% alginate beads (diameter ~3 mm, Mansanto Keltone® LVR) at 20x10⁵ cells/ml. Cell-seeded beads were cultured in high-glucose DMEM with either 10% FBS or the growth factor (GF) combination of 100 ng/ml IGF-1 + 100 ng/ml OP-1. At selected times in culture, alginate beads were depolymerized in 55 mM NaCitrate for 5 minutes, mixed gently until dissolved, spun down, rinsed in PBS, spun down, and the cells resuspended in high-glucose medium to 1x10⁶ cells/ml. AFM Indentation: The cell suspension was then deposited onto a silicon substrate with square pyramidal wells, ranging from 15-22 µm on a side, to immobilize single cells for AFM nanoindentation. The cells were moved into the wells using a Si₃N₄ atomic force microscope (AFM, Picoforce, Veeco Instruments) cantilever probe tip (Veeco Instruments, spring constant, k ~ 0.06N/m, end radius, R₀ ~ 40nm). For cell indentation, a nano tip, with a nano tip velocity of 1 µm/s were used to obtain normal force vs. indentation depth (distance) curves. Standard AFM Si₃N₄ probe tips (R₀ ~40nm) and silica colloidal probe tips (R₀ = 2.5µm) were used for indentation on the nano- and micron-scales.

PCM Characterization: PG and collagen content were measured via dimethyl methylene blue dye binding and hydroxyproline assays, respectively. Optical micrographs of cells released at each time point were obtained to measure the diameter of the cell and to aid in estimating PCM thickness.

Theoretical Modeling: Finite element analysis (FEA; ABABUSAC Inc.) was used to estimate the stiffness of freshly isolated cells with no PCM, accounting for cell-boundary conditions, cell curvature, and probe tip geometry. Using these values, a simple FEA model of a cell with an elastic shell of PCM was used to estimate the modulus of the PCM, based on indentation data for cells and their newly developed PCM.

RESULTS: Biochemical Composition: Cells supplemented with GF from days 7-28, compared to FBS, showed higher PG content (Fig. 1a), though collagen levels were more similar (Fig. 1b). Histological staining for GAG and collagen showed a larger diameter PCM with time for GF compared to FBS (not shown). Nanoindentation of Freshly Isolated Cells: A higher force throughout indentation was measured with the micro tip compared to the nano tip (Fig. 2). Nanoindentation of FBS Cells: The force vs. indentation curves measured with both the nano and micro tips showed a slight stiffening with increasing time in culture (Fig. 3). However, even at day 28 cells+PCM were significantly less stiff than freshly isolated cells as shown by the lower force generated at the same indentation depths. Nanoindentation of GF Cells: GF cultured cells showed a large increase in stiffness with time in culture using micro tips (Fig. 3) and nano tips (not shown) compared to that of parallel FBS cultured cells. Control, i.e. day 0, the day 21 cells probed with the micro probe tip exhibited a slightly lower stiffness (Fig. 3). However by day 28, the cell-PCM complex appeared stiffer at all indentation distances. FEAA Modeling: Using the known geometry of the micro tip and well, along with no-friction cell-wall boundary conditions, a comparison of day-0 force-distance data (Fig. 2) to model predictions gave an estimated cell Young’s modulus E = 3.0 kPa using a Poisson’s ratio of 0.4 [5]. Using this value of E, FEA including the PCM shell and measured PCM thickness ~3.65±8.87 µm (n=18 cells) gave a shell (PCM) modulus of 0.1kPa. In marked contrast, when compared to day 28 GF cultured cells, FEA predicted a much higher PCM modulus of 4.15kPa. FEA also indicated that the cell compressed more, at any indentation, when surrounded by the 0.1kPa PCM compared to the 0.01kPa PCM.

DISCUSSION: Freshly isolated (day 0) cells, probed at two length scales using the nano and micro tips, showed a higher stiffness with the micro tip (Fig 2). This is due to the likelihood that the nano tip may be sensing the underlying cytoskeleton or organelles, whereas the micro tip, with its larger contact area, may be measuring overall cell bulk properties. For both tips, a low stiffness was observed for the FBS fed cells+PCM; this may be due to the loose arrangement of PGs and collagen in the newly synthesized PCM, which may have properties that are much softer than that of the cell alone. However, cells cultured in GF showed much stiffer properties (Fig. 3), consistent with previous observations [6] that such GF stimulation results in a significant increase in PG synthesis by day 21 in alginate. While total collagen content was not very different with GF vs. FBS in our study, GF may also stimulate faster maturation of the collagen network and PG-collagen integration within the PCM, which would be consistent with increased stiffness.


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Figure 1. (a) GAG content of PCM was higher for cells supplemented with GF compared to FBS. (b) Collagen content was similar for both cell cultures. Pooled data using 3 beads/condition/time point.

Figure 2. Indentation on freshly isolated chondrocytes (day 0) showing higher force with micro (n=17 cells) vs. nano tip (n=25 cell). mean±SD.

Figure 3. Indentation (mean ± SD) on loading of individual cell + PCM released from alginate at days 21 and 28 from culture in GF vs. FBS, and compared to freshly isolated chondrocytes (day 0), all using the micro tip.