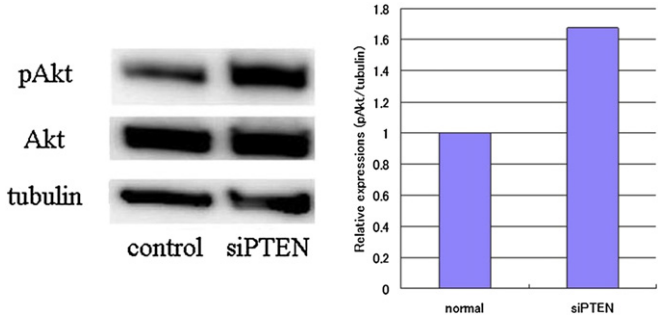

Figure 4. Phosphorylation of Akt after treated with t-BHP Normal chondrocytes.

241
ENCAPSULATION OF CHONDROCYTES IN PHYSIOLOGICALLY-STIFF AGAROSE

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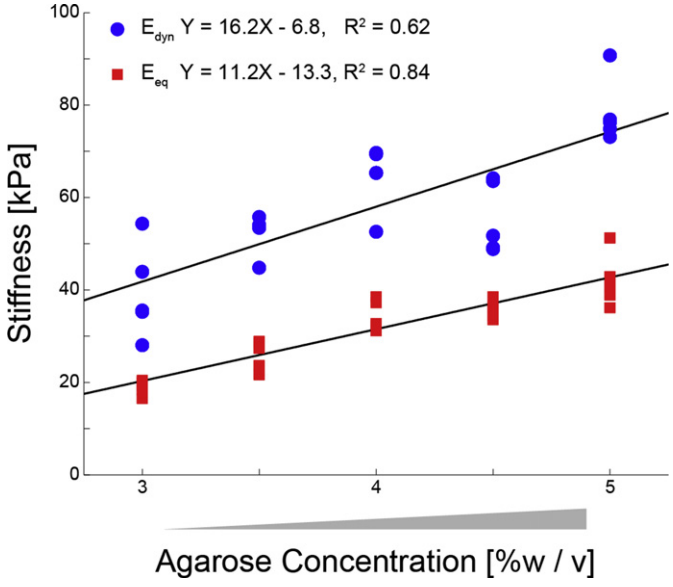
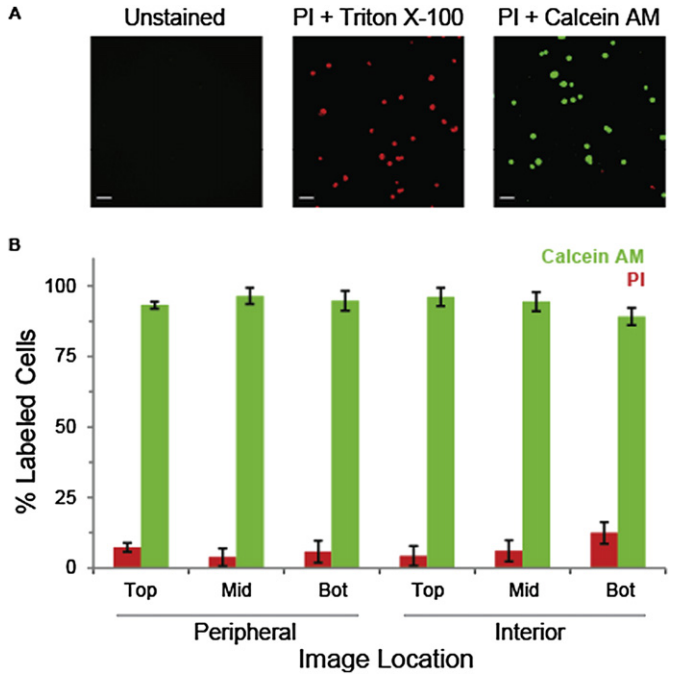
Purpose: The cellular microenvironment contains insoluble matrix, soluble signals and nutrients, and extracellular water, to maintain cell and tissue homeostasis. Articular chondrocytes reside within a pericellular matrix (PCM) defining their immediate surroundings and providing both molecular and mechanical signals. One biomechanical cue, which alters cellular phenotype is stiffness, which is provided by the PCM. Differences in PCM stiffness have been found between normal and osteoarthritic chondrons, with measured values of ~25-200 kPa. The objective of this study was to test the hypothesis that cultured chondrocytes can be encapsulated in high-concentration agarose gels of physiological stiffness. Our novel results suggest that agarose can provide a 3D microenvironment capable of both maintaining cellular viability and mimicking the PCM stiffness of both normal and OA tissue.
Methods: Cylindrical agarose gels were prepared in PBS and maintained at 4°C until mechanical testing (<48 hours) when samples were compressed in increments of 4% nominal compression followed by 2 hours of stress-relaxation through 16% final compression. Nominal stresses were calculated by dividing the load by the measured cross sectional area. Linear regression was used to determine dynamic and equilibrium stiffness values from the stress (peak and equilibrium) and strain data.
SW1353 chondrocytic cells were grown in DMEM with 10% calf serum and antibiotics. Cells were trypsinized, counted, pelleted (600 x g, 7 minutes), and resuspended in media at 3 x 10^6 cells / mL (11X). Agarose was dissolved in PBS at 1.1X (4.4% w / v). Cell suspensions and agarose solutions were mixed prior to casting at 25°C for 10 minutes before incubation in tissue culture (37°C, 5% CO2, DMEM with 10% serum and antibiotics). Viability was assessed after 24 hours in tissue culture by incubating cells in 50 μg / mL propidium iodide (PI) and 5 μM Calcein-AM in PBS for 1 hour. Samples were imaged in PBS on a Leica SP5 upright confocal microscope using a 25X submersible objective. Cells were counted using Leica software following thresholding. Statistical analyses were performed with an a priori significance level of α=0.05.
Results: A significant relationship was found between the stiffness and gel concentration (Figure 1, Dynamic: r = 0.79. Equilibrium: r = 0.92 Both $p < 0.001$, $n = 5$). We achieved agarose equilibrium stiffness values as large as 51.3 kPa (Figure 1). At 4.0% agarose, we found equilibrium moduli of 34.3 +/- 1.65 kPa, and at 4.5% agarose, we found equilibrium moduli of 35.7 +/- 0.95 kPa. Imaging revealed high viability (Figure 2), and 95% confidence intervals for the number of PI positive nuclei upon detergent addition were not location-specific indicating that the methods resulted in an even spatial distribution of cells. Furthermore, we found no viability differences between regions. Overall viability was 94.0 ± 1.0% (n=3).
Conclusions: These data demonstrate the feasibility of encapsulating chondrocytic cells in agarose of physiological stiffness. Because the stiffness of the pericellular matrix is affected by osteoarthritis these methods provide a foundation for both improving the physiological relevance of in vitro culture systems and applying mechanical loads to chondrocytes.





242
REVERSAL OF THE NEGATIVE EFFECTS OF TGFβ1 ON HUMAN ARTICULAR CHONDROCYTES BY PKCδ INHIBITION WITH ROTTLERIN

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Purpose: Rottlerin (ROT), a natural plant polyphenol, displays potent and highly selective inhibition of PKCδ. Extensive genomic, proteomic, and cell signaling studies revealed that ROT may excert anti-oxidant and anti-inflammatory effects but the mechanisms responsible are not known. Other studies have suggested that both PKCδ and TGFβ1 signaling can modulate the expression of extracellular matrix proteins (ECM) as well as ROS production. However TGFβ1 and PKCδ signaling interactions in human chondrocytes have not been thoroughly