Analysis of Chondrocyte Metabolism In Vitro Utilizing Different Culture Methodology

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Introduction
Osteoarthritis (OA) is a painful and debilitating disease that is the number one cause of disability in the United States. Many in vitro models of OA have been used to better understand this disease, including cartilage tissue explant culture, chondrocyte monolayer culture, and chondrocyte 3-D culture. The metabolism of chondrocytes in monolayer cell culture has been shown to be different from chondrocytes in cartilage in vivo. Chondrocytes in monolayer cell culture lose the classic cartilage phenotype (aggrecan and collagen II expression) and de-differentiate into a more fibroblastic phenotype (collagen I expression). The process of de-differentiation occurs within a few passages and may not occur in cell culture. It is not known how the process of de-differentiation affects basal chondrocyte metabolism or the chondrocytes’ responses to various stimuli often used to mimic osteoarthritis in vitro. Therefore, it is possible that the data collected using cells propagated in monolayer cell culture is unsuitable for the study of osteoarthritis and normal cartilage metabolism. Chondrocyte 3-D culture has been shown to rescue the cartilage phenotype, with respect to collagen expression, but it is not clear how 3-D culture affects basal and cytokine stimulated chondrocyte metabolism. Therefore, more data needs to be collected focusing on the metabolic responses of chondrocytes in different types of in vitro culture to stimuli (like interleukin (IL-1β) commonly used for the analysis of cartilage metabolism and to model osteoarthritis. Once the metabolic responses of chondrocytes cultured under different in vitro conditions are better understood, the utility of various in vitro models to study specific aspects of cartilage metabolism in health and disease can be determined.

Objectives
- Characterize the temporal basal and IL-1β-stimulated production of nitric oxide (NO), prostaglandin E2 (PGE2), matrix metalloproteinase (MMP)-2, MMP-3, MMP-9 and MMP-13 of chondrocytes in cartilage explant culture, monolayer culture, and 3-D agarose culture.
- Compare the production of MMPs, NO, and PGE2 in response to IL-1β between cultured chondrocytes and cartilage explant tissues. Identify the time point during culture at which significant differences in the metabolism of chondrocytes in cell cultures and cartilage explant cultures occur in vitro.

Hypotheses
- The basal and IL-1β-stimulated production of MMP-2, MMP-13, NO, and PGE2 will be significantly different between cartilage explant culture, monolayer culture, chondrocyte 3-D culture, and tissue explant culture.
- Chondrocytes in 3-D culture will behave more like those in cartilage explant culture than those in monolayer culture with respect to the metabolic factors studied.

Methods
Tissue harvest and culture: Full thickness articular cartilage was harvested from the humeral heads of 6 skeletally mature canine cadavers euthanized for reasons unrelated to this study. Tissue explants (CE) were prepared sterilely using a 4 mm dermal biopsy punch. Two explants were cultured in a 24 well plate in 1 ml of supplemented DMEM media, with or without (NEG) recombinant human (rh)IL-1β (100 ng/ml). For monolayer (M) and 3-D (3D) cell culture, chondrocytes were isolated from cartilage tissue by collagenase digestion. Isolated chondrocytes were counted and tested for viability by trypan blue exclusion assay, and 1 ml (70,000 cells/ml) was used to seed 24 well plates (6 wells per dog, 2 well culture). Cells were either used for monolayer cell culture (3 wells per dog) or used with rhIL-1β (9 (NEG), 10, or 100 ng/ml); used to create 3D cell culture constructs (3 wells per dog) and cultured with rhIL-1β (0, 10, or 100 ng/ml); or passaged (3D constructs) 4% agarose solution was created in DMEM media and kept liquid at 37°C. One well of cells was mixed with agarose and allowed to gel at 4°C to make a final 2% agarose construct for culture. The gel constructs were placed in a 24 well plate and cultured in 1ml of media as described above. All 3 culture types were incubated at 37°C with 5% CO2 and 10% humidity for 9 days of culture. Media was changed and collected every 3 days and stored at -20°C for later testing. On day 9, cells, tissues, and 3-D constructs were digested with papain solution and stored at -20°C for DNA content testing.

Data was analyzed for significance by One-Way ANOVA with significance set at p<0.05.

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Discussion
This study indicates that there is a significant change in the metabolism and phenotype of chondrocytes in monolayer cell culture compared to tissue explant culture. The basal production of MMPs and inflammatory markers and the responses to cytokine stimulation are notably different between the two culture types. The loss of nitric oxide production, the increase in MMP-2 and MMP-13 production, and the lower fold-increase of MMP-13 in response to IL-1β indicate a loss of chondrocytic phenotype, which is progressive with cell culture passage. Although it was hypothesized that the 3-D culture would restore the phenotype by allowing the chondrocytes to retain their shape, the phenotype was not restored in the time allowed for this study. This indicates that the phenotypic change is not simply a result of cell shape change. It is possible that over time, the 3-D culture could develop an extracellular matrix, and restore the chondrocytic phenotype observed in tissue explant culture.

Conclusions
- The basal and IL-1β stimulated metabolic responses of chondrocytes are significantly different between tissue explant culture and monolayer cell culture.
- 3-D culture did not allow for recovery of chondrocyte phenotype after monolayer culture as assessed during the time frame of this study.