Chondrocytes from Human, Horse, Camel, Dog, and Cat Articular Cartilage Propagated on Microcarrier Spinner Culture Respond to Pro-inflammatory Stimuli

[1]Grzanna, MW; [1]Heinecke, LF; [1,2]Au, AY; [1]Ownby, SL; [1]Mrozinski, AC; [1,3,4]Frondoza, CG
[1]Nutramax Laboratories, Inc., Edgewood, MD, [2]Syracuse University, Syracuse NY, [3]Mississippi State University, Mississippi State, MS, [4]Johns Hopkins University, Baltimore, MD
cfrondoza@nutramaxlabs.com

INTRODUCTION:

Chondrocyte cultures are used to study proliferation, differentiation, metabolism, and response to various stimuli including cytokines. However, chondrocytes in monolayer culture lose their original phenotype and become fibroblastic. This change may alter behavior and response to stimuli. Previous studies have shown that collagen microcarrier spinner culture favors human chondrocyte proliferation while maintaining their phenotype [1]. Here, we hypothesize that chondrocytes from different species can proliferate in collagen microcarrier spinner culture and respond to pro-inflammatory stimuli resulting in production of mediators such as prostaglandin E2 (PGE_2). Inflammation plays a critical role in the pathogenesis of osteoarthritis (OA). Excessive production of inflammatory mediators is associated with pain and cartilage breakdown in OA (2). Like humans, many species including horses, camels, dogs, and cats suffer from OA. The chondrocyte microcarrier spinner culture offers a biomechanical model for evaluating the inflammation pathway and agents that could modify this pathway.

METHODS:

Chondrocytes from human, horse, camel, dog and cat cartilage were grown as monolayer for up to four passages. Cells were then seeded onto solid collagen 100-400µm microcarriers from bovine corium. Chondrocyte ($4x10^3$ cells/cm^2) microcarrier cultures were propagated at 60 rpm, 37ºC, 5% CO_2 for 7 days and were visualize by phase-contrast and TEM. Production of collagen and aggrecan was analyzed by immunofluorecence. Chondrocyte cultures (10ml) were incubated in micro-spinner flasks with control media alone, or with IL1β (10, 50ng/mL) and TNFα (1ng/ml) for 24 hrs. PGE_2 production was measured by ELISA.

RESULTS:

Human, horse, camel, dog, and cat chondrocytes in microcarrier spinner culture proliferated with ease (Figure 1 top and lower panels). Microcarriers formed aggregates by seven days and produced extracellular matrix materials (ECM) (Figure 1 top panel, 3 frames and lower panel, first and second frame). TEM revealed chondrocytes with well defined ultrastructure on the surface of microcarriers (Figure 1, lower panel, third frame). Chondrocytes and their ECM immunostained intensely for type II collagen and aggrecan (Figure 2, left and right panel respectively). Chondrocytes from five different species responded to cytokine activation with 2 to 40,000 fold increase in PGE_2 production (Figure 3, $p<0.001$).

DISCUSSION:

The key finding is that the collagen microcarrier spinner cultures can be used to propagate chondrocytes from different species. That chondrocytes seeded on collagen microcarriers respond to pro-inflammatory stimuli indicates the maintenance of their ability to respond to biologic stimuli. The dynamic biomechanical environment appears to recapitulate what chondrocytes encounter in the joint. This model provides a useful tool to study molecular targets in the inflammatory cascade and to identify agents that could affect these targets.

Figure 1. Top panel, three frames: Chondrocyte-seeded microcarriers following 7 day culture. Phase contrast micrographs from human, feline, equine, canine and camel chondrocyte chondrocytes. Bar = 50µm. Lower panel, third frame: TEM of canine chondrocyte seeded-microcarrier.

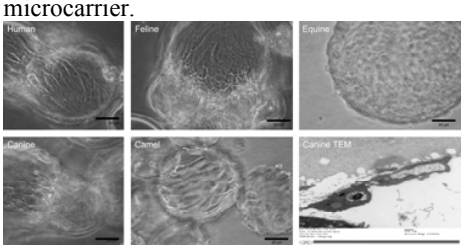


Figure 2. Human chondrocyte immunofluorescence: (Left panel) Type II collagen; (Right panel) Aggrecan. FITC (green). Nuclei are stained blue with DAPI.

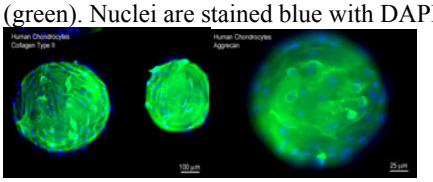
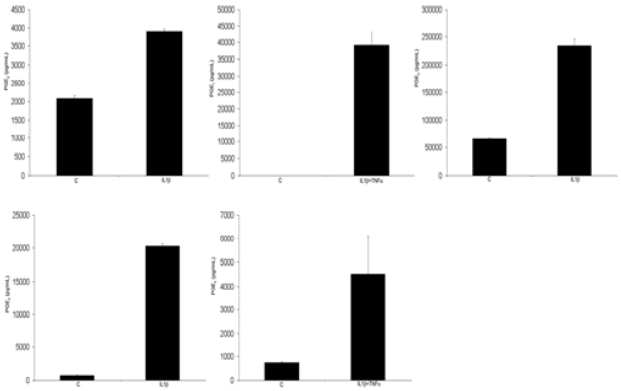


Figure 3. Chondrocyte-seeded microcarriers respond to activation by cytokines IL1β and TNFα by increased production of PGE_2 ($p<0.001$), ANOVA Tukey Analysis



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