Modulation of Prostaglandin E-2 Production by Chondrocytes on Solid Collagen Microcarriers

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Introduction: Prostaglandin E-2 (PGE-2) plays a critical role in the pathogenesis of osteoarthritis (OA). This debilitating joint disease is characterized by pain and cartilage breakdown. Chondrocytes synthesize PGE-2 and other pro-inflammatory molecules that mediate cartilage destruction. Chondrocyte models have been used to evaluate PGE-2 production in response to various stimuli including cytokines, microbial products, and chemical and physical agents. However, chondrocytes in monolayer culture lose their original phenotype by shifting from producing type II to type I collagen and from high to low molecular weight proteoglycans. This phenotypic change alters chondrocyte behavior and their response to stimuli. Previous studies showed that bioreactor culture systems favor chondrocyte proliferation while maintaining their phenotype [1]. We evaluated whether chondrocytes propagated in dynamic microcarrier spinner culture can be activated by interleukin-1 beta (IL-1β) to produce PGE-2. The study also evaluated whether this activation can be blocked by natural products: avocado/soybean unsaponifiables (ASU), chondroitin sulfate (CS), and glucosamine hydrochloride (Glu) [2-3].

Methods: Canine articular chondrocytes expanded in monolayer for 21 days were seeded onto collagen microcarrier beads ($4x10^3$ cells/cm^2). The cell-seeded microcarriers were propagated at 60 rpm, 37ºC, 5% CO_2 for 14 days [1]. Aliquots were incubated in 25 ml spinner flasks for 24 hrs with: (i) control media alone or (ii) the combination of NMX 1000™ avocado/soybean unsaponifiables (25µg/mL), TRH122® chondroitin sulfate (20µg/mL), and FCHG49® glucosamine hydrochloride (11µg/mL) supplied by Nutramax Laboratories, Inc., Edgewood, MD. Next, the cell-seeded microcarriers were activated with IL-1β for 24 hrs. The supernatant was assayed for PGE-2 content. Chondrocytes were analyzed by phase-contrast microscopy and by immunofluorescent staining for type II collagen.

Results/Discussion: Chondrocytes attached with ease to microcarriers and displayed well-defined spherical morphology. By day 7, the chondrocyte-seeded microcarriers formed aggregates. Extracellular matrix (ECM) material was visible around and between microcarriers (Figure 1, left panel). By day 14, larger aggregates with abundant and dense ECM were observed
(Figure 1, right panel). Continued production of type II collagen, which constitutes the major component of hyaline cartilage, was confirmed by immunostaining. Activation of chondrocyte-seeded microcarriers at passage 3, 4, and 6 with IL-1β showed similar responsiveness to the cytokine. There was a 79%, 65%, and 160% increase in PGE-2 levels at Passage 3, 4, and 6 respectively (Figure 2) when compared to non-activated controls. Pretreatment with the combination of ASU, CS, and Glu reduced PGE-2 levels to approximately 56% below IL-1β activated levels (Figure 3).

Figure 1. Chondrocyte-seeded microcarriers

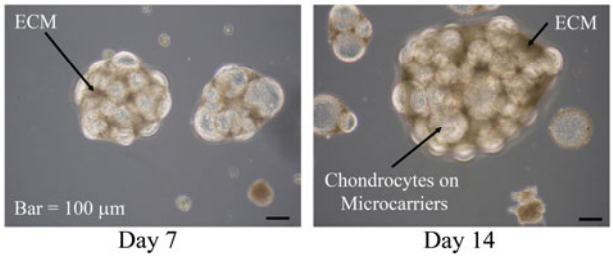


Figure 2. PGE-2 levels produced in chondrocyte-seeded microcarriers at different passages

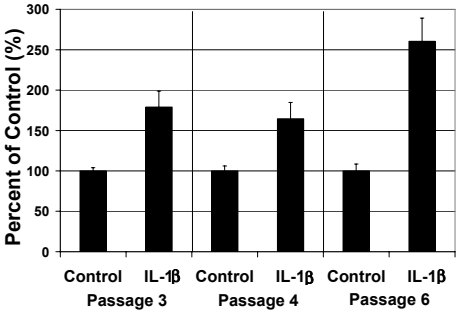
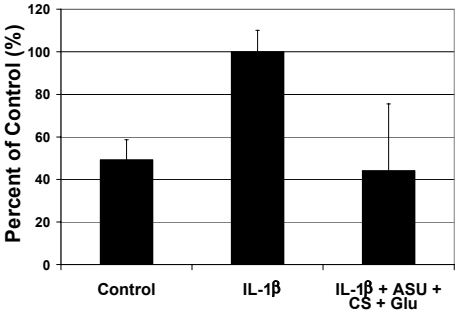


Figure 3. PGE-2 levels produced in chondrocyte-seeded microcarriers incubated with ASU, CS, and Glu



Conclusions: The present study demonstrates that the microcarrier spinner culture system can be used to evaluate chondrocyte responses to pro-inflammatory stimuli and identify agents that can modify these responses. The dynamic condition in the microcarrier spinner bioreactor appears to recapitulate the biomechanical environment that chondrocytes encounter in the joint. The microcarrier spinner culture system may serve as a useful tool to evaluate the potential anti-inflammatory properties of natural products. Using this culture system, we observed that the combination of ASU, CS, and Glu effectively blocks activation of the inflammatory pathway.

References:

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