

CO-CULTURE CHONDROCYTE SYSTEM: AN ATTEMPT TO IMPROVE CHONDROCYTE PROLIFERATION. Madaí A. Gomez-Camarillo^{1,2}, Juan B Kouri¹. ¹Centro de Investigación y de Estudios Avanzados-Instituto Politécnico Nacional (CINVESTAV-IPN), Mexico. ²Universidad Autónoma de Tlaxcala, México.

Introduction: Osteoarthritis (OA) has become a public health problem. Adult articular cartilage is a tissue with limited capacity to respond to injuries and low potential for self-repair. Many strategies have been tested trying to resolve this problem. One of these strategies, the chondrocyte culture, has been tested in different systems, in monolayer^[1], agarose gels^[2], collagen gels^[3] and calcium alginate^[4]. The aim of this study was to test a co-culture system that enhances the stimulation of chondrocyte proliferation. **Material and Methods:** We propose a co-culture system that uses a monolayer of dedifferentiated chondrocytes as a source of mitotic factors, co-cultured with chondrocytes, encapsulated in alginate beads previously stained with carboxyfluorescein diacetate succinimidyl ester (CFSE), a stain that allows tracing cell division by flow cytometry. We used a control system with stained CFSE chondrocytes encapsulated in alginate beads cultured without a monolayer of dedifferentiated chondrocytes. Chondrocyte division was assessed by flow cytometry; morphologically, by identification of mitosis phases, and apoptosis TUNEL test; and by chondrocyte phenotype, collagen type II expression. **Results:** Morphologically, all mitosis phases were detected and a very low percent of apoptotic cells was found in the co-culture system (Fig.1). The positive expression of collagen type II in samples of chondrocytes from the co-culture system demonstrated that chondrocytes resulting from this system retain their phenotype (Fig.2). Flow cytometry analysis revealed that the chondrocytes encapsulated in alginate beads co-cultured with dedifferentiated chondrocyte monolayer show a significantly higher proliferation compared with chondrocytes within alginate beads cultured without dedifferentiated chondrocyte monolayer (Fig.3). **Discussion:** Many chondrocytes cultured systems has been tested, however to the best of our knowledge, this is the first report on chondrocytes proliferation in a co-culture system. The substantial increase of chondrocytes proliferation found in this work; open the possibility to study this phenomenon in OA cartilage.

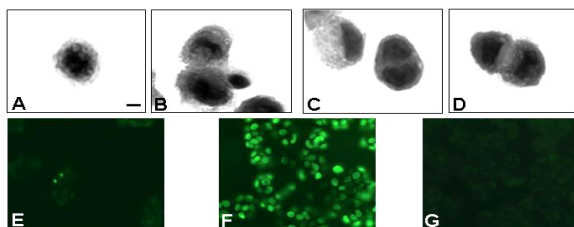


Fig. 1. A: Prophase, B: Metaphase, C: Anaphase, D: Telophase, E: TUNEL Co-culture, F: TUNEL Positive Control, G: TUNEL Negative Control. Scale barr 10 μ m.

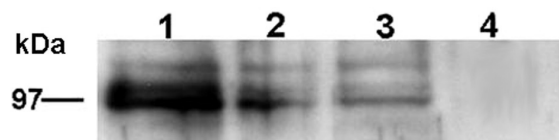


Fig. 2. Collagen II expression. 1. Positive Control Normal Cartilage; 2. Co-Culture Chondrocytes; 3. Control Culture Chondrocytes; 4: Negative Control HeLa Cells.

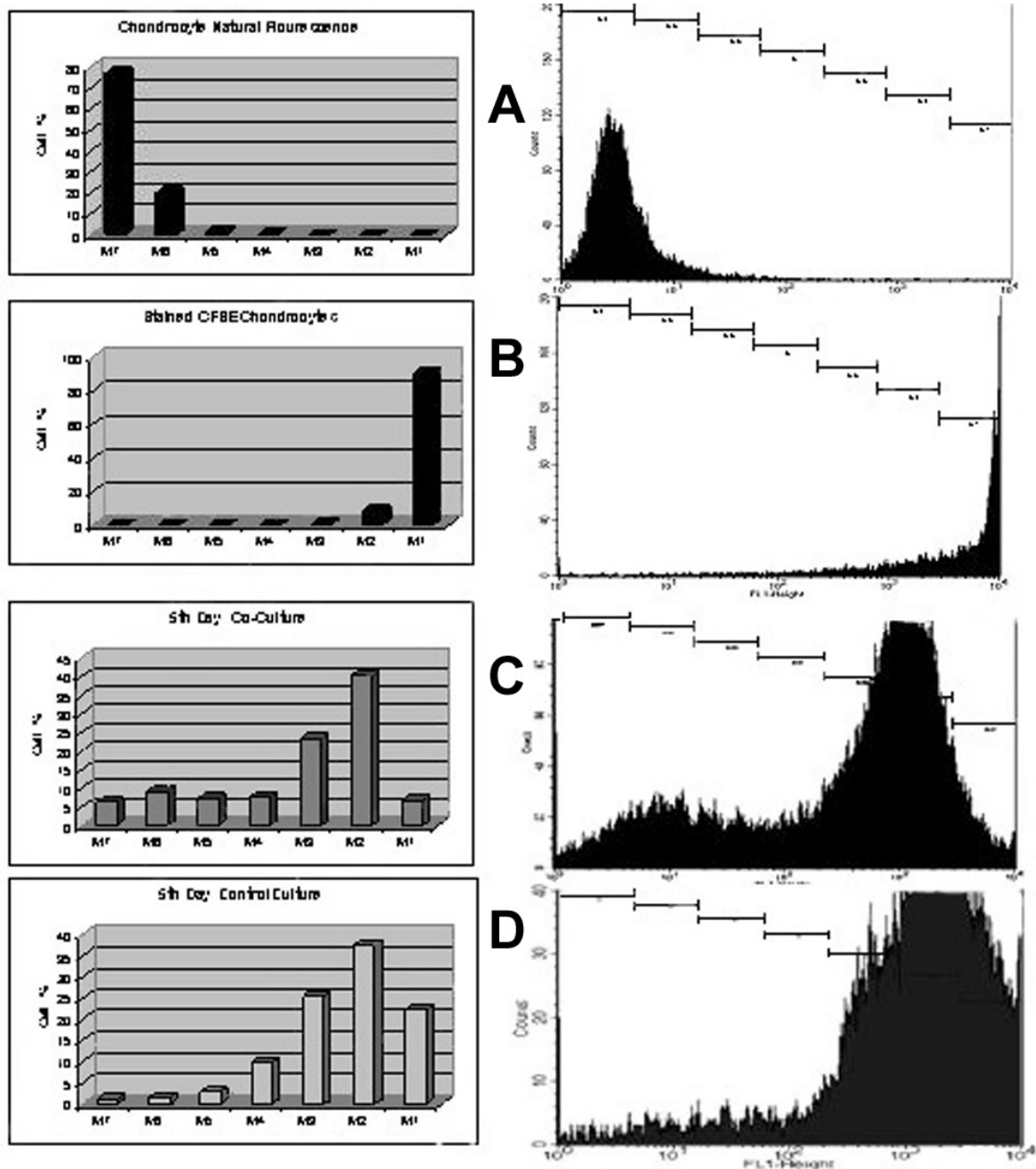


Fig. 3. A: Chondrocyte natural fluorescence; B: Stained chondrocyte with CFSE; C: Stained chondrocytes after 5 days of co-culture; D: Stained chondrocytes after 5 days of control culture. The flow cytometry histograms and graphics were divided into seven zones (M1-M7), M1 represents the zone where CFSE stained cells with high fluorescence intensity were placed and M7 the zone where cells without fluorescence were placed; each consecutive zone corresponds to two-fold decrements in cellular fluorescence intensity. Each zone represent a new cell division.

References:

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- [2] Aidelotte MB et al., Orthoped Res Soc 1991, 16: 26-31
- [3] Buschmann MD et al., J Orthoped Res 1992, 10:745-748
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