

STUDY OF THE *IN VITRO* CONDITIONS PROMOTING HYPERTROPHIC DIFFERENTIATION OF OA ARTICULAR CHONDROCYTES



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Objective. Hypertrophic differentiation of chondrocytes is an important feature in OA cartilage which is involved in extracellular matrix mineralization. This study aimed to develop a new culture model for studying chondrocyte hypertrophic differentiation *in vitro*.

Methods. Articular OA chondrocytes were cultured for 28 days in monolayer or in alginate beads in different culture medium (1% Insulin Transferrin, Selenium and BSA, 2% Ultrosor G or 10% Fetal Bovine Serum). DNA was quantified by fluorimetry. The expression of gene characteristics of chondrocyte phenotype (AGG, COL2A1, SOX9), pre-hypertrophic differentiation (ihh, PTH-R, PTHrP), hypertrophic differentiation (COL10A1, cbfa1), matrix mineralization (Ank, ENPP1, CILP, TG2, FXIIIa) or vascularization (VEGF) was evaluated by RT-PCR. Alkaline phosphatase activity, transglutaminase activity and 5' phosphodiesterase activity of NTPPPH were quantified by specific enzymatic methods.

Results. In 10% FBS chondrocytes expressed significantly more hypertrophic (PTHR, PTHrP, ihh, COL10A1 and cbfa1) and matrix mineralization genes (TG2, FXIIIa, CILP, ENPP1 and Ank) in alginate beads than in monolayer (Figure 1). Whatever the culture system (alginate beads or monolayer), chondrocytes cultured in 10% FBS expressed significantly more hypertrophic and matrix mineralization genes like COL10A1 than chondrocytes cultured in 1% ITS+ or 2% UG (Figure 2).

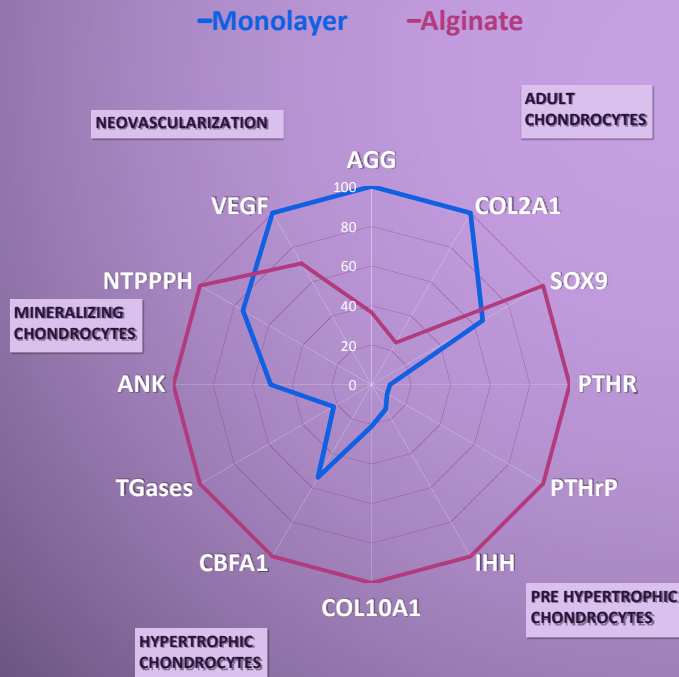


Figure 1: Expression of genes and enzymatic activities of chondrocytes after 21 days of culture in 10% FBS media., n=3.

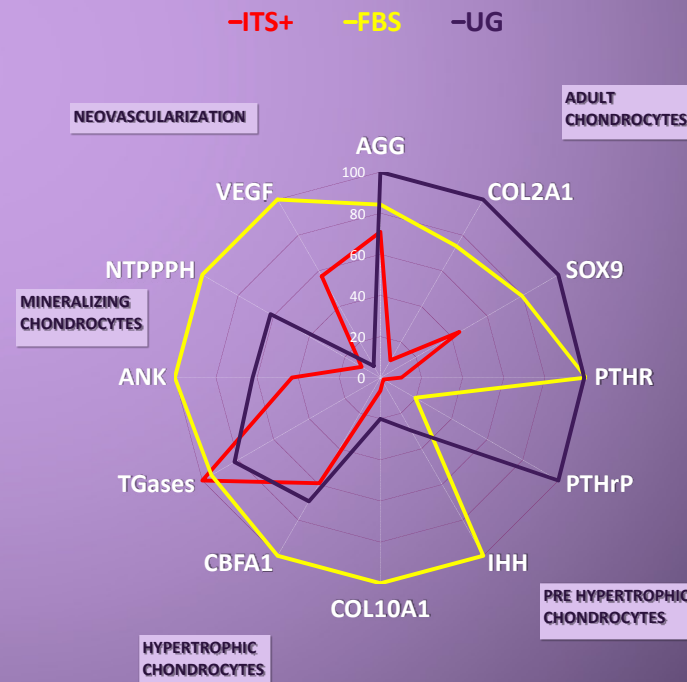


Figure 2: Expression of genes and enzymatic activities of chondrocytes after 21 days of culture in alginate beads, n=3.

Conclusions.

The expression of hypertrophic markers in articular chondrocytes is upregulated in fetal bovine serum (FBS) and alginate beads culture conditions, suggesting that cartilage neovascularization could be a factor of chondrocyte hypertrophic differentiation in OA cartilage.