PHENOTYPE SHIFT OF CHONDROCYTE MONOLAYER CULTURES IN POLYSTYRENE SURFACE IS ASSOCIATED WITH INCREASED ADHESIVE ABILITY

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Introduction: A major obstacle in chondrocyte based therapy for cartilage repair is the limited availability of cells that maintain their original phenotype. Propagation of chondrocytes as monolayer cultures on polystyrene surfaces is used extensively for amplifying cell numbers. However, chondrocytes undergo phenotypic shift when propagated as monolayers in polystyrene surfaces. They switch from expressing their original type II collagen in hyaline cartilage to type I collagen which is characteristic of fibroblastoid cells. They also shift from expressing high molecular weight proteoglycans (called aggrecan) to low molecular weight proteoglycans. Little is known about the effect of this shift on adhesion properties. In cartilage, chondrocytes produce adhesion molecules that enable these cells to anchor, integrate and communicate with their environment. Here, we tested the hypothesis that the loss of cartilage phenotype is accompanied by a change in adhesive function. This study will help define culture conditions that favor maintenance of the cartilage phenotype.

Materials and Methods: Bovine chondrocytes were propagated on polystyrene flasks for two weeks. Supernatant was assayed for type I collagen by ELISA. Gene expression was analyzed by reverse transcription polymerase chain reaction (RT-PCR) using primers specific for type II and I collagen, aggrecan and S14 housekeeping gene. Adhesion properties were evaluated under laminar flow using the Glycotech® flow chamber. Chondrocytes (5×10^4) or L929 fibroblasts were incubated on glass slides for 5 hrs. at 37° C, 5% CO_2. Three shear stress measurements were made for each specimen. The effect of shear stress, e.g. cell deformation and cell loss, was determined by microscope image capture every 5 min.

Results: Chondrocytes attached and proliferated on polystyrene surfaces. They appeared elongated and fibroblastoid with continued passage. They expressed decreased transcript levels of type II collagen and aggrecan (Figures 1 and 2). This was accompanied by a time dependent increase in type I collagen expression (Figures 1 and 3). Chondrocytes increased production of type I collagen: passage 1=20 $\pm$ 4.5 ng/ml, passage 4=30 $\pm$ 2.3 ng/ml, passage 8=45 $\pm$ 6.0 ng/ml. The pattern of dedifferentiation was paralleled by enhanced adhesive properties. Chondrocytes required more than 124 dynes/cm^2 to detach from the surface by passage 3, similar to the adhesive properties of fibroblasts (Figure 4).

Discussion and Conclusion: The present study demonstrates that the dedifferentiation profile indicated by a shift from chondrocytic to fibroblastoid gene expression is associated with increased adhesive ability. Our data indicates that analysis of phenotype markers as well as adhesive properties may serve as useful indicators to identify culture conditions that may help preserve the chondrocyte phenotype and function.

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Figure 1

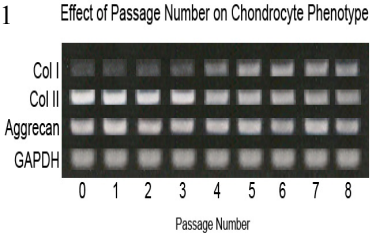

Figure 2

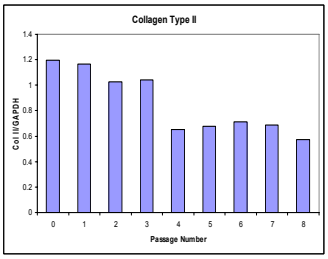

Figure 3

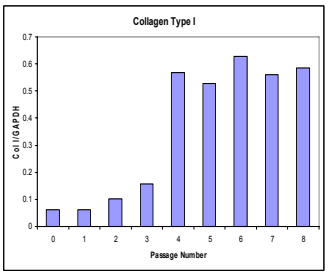

Figure 4