

ISOLATION AND EXPANSION OF HUMAN ARTICULAR CHONDROCYTES IN MONOLAYER CULTURE INDUCES A PROINFLAMMATORY CYTOKINE PROFILE

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INTRODUCTION: For autologous chondrocyte transplantation (ACT), chondrocytes are expanded in monolayer culture. During this expansion chondrocytes may dedifferentiate into fibroblast-like cells, characterised by e.g. decreasing type II collagen expression. However, little is known about changes in the expression of cytokines involved in cartilage anabolism and catabolism during this process. Since the production of a good quality cartilage might be enhanced through the transplantation of chondrocytes with an anabolic cytokine profile, we analysed the cytokine expression patterns of chondrocytes, which were expanded in monolayer culture and subsequently recultivated in alginate beads.

METHODS: Human articular chondrocytes were obtained from cartilage biopsies (n=6). mRNAs encoding type I and II collagen, IL-1, -4, -10, -17, -18 and BMP-2 and -4 were evaluated by quantitative RT-PCR in one aliquot. Expression patterns were established for freshly isolated chondrocytes, chondrocytes after primary expansion (~2-4 population doublings) in monolayer culture, and for expanded chondrocytes recultivated in alginate beads.

RESULTS: After primary expansion in monolayer cultures, increased IL-4, IL-18 (Fig 1), type I collagen and BMP-4 transcription in comparison with freshly isolated chondrocytes was observed. At the same time, type II collagen, BMP-2, IL-10 and IL-17 mRNA transcription decreased. Recultivation of the monolayer expanded cells in alginate resulted in the reexpression of type II collagen, BMP-2 and IL-10 whereas IL-18 and BMP-4 expression was reduced.

DISCUSSION & CONCLUSIONS: This study shows that in chondrocytes cultivated in monolayer culture IL-10 expression is strongly reduced, while IL-18 expression is increased. IL-10, is known for its IL-1 antagonizing properties; it protects chondrocytes from destructive and catabolic IL-1 effects. IL-18 induces proinflammatory and catabolic responses in chondrocytes and contributes to cartilage degeneration. These results suggest that monolayer culture of chondrocytes not only results in the dedifferentiation of these cells (as shown by the shift from type II towards type I collagen production), but also leads to the establishment of a proinflammatory phenotype. Combined this may lead to a suboptimal formation of cartilage upon transplantation of these cells. Therefore, it is of great interest that the 3D-recultivation of monolayer-expanded chondrocytes not only results in the restoration of the chondrogenic phenotype, but also results in a reduced expression of proinflammatory cytokines.

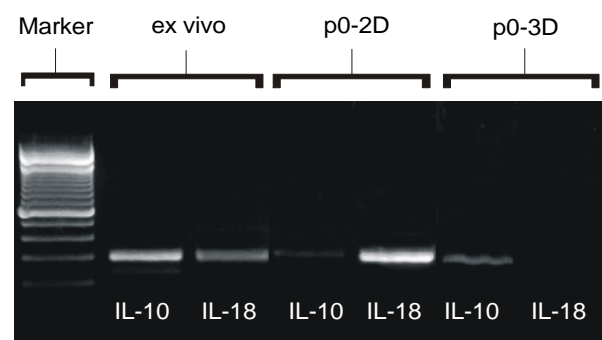


Fig 1. IL-10 and IL-18 expression of human articular chondrocytes seeded in monolayer culture and alginate beads. Ex vivo = freshly isolated human articular chondrocytes. p0-2D = chondrocytes after expansion in primary monolayer culture. p0-3D = expanded chondrocytes from p0-2D recultivated in alginate.