INTRODUCTION:
Autologous chondrocyte implantation (ACI) is a widely used method for treating focal articular cartilage lesions. Despite encouraging clinical follow-up data, there are still some concerns whether chondrocytes that undergo a number of passages maintain their phenotype, proliferative activity, and the ability to synthesize and properly assemble newly formed extracellular matrix (ECM). Also in question is if these cells need to be stimulated with growth factors to remain metabolically active. Previous studies, Gigante et al. (1) analyzed chondrocytes proliferation and, after ACI implantation by immunocytochemistry and found that prior to implantation these cells maintain the differentiated phenotype of mature chondrocytes and produce type II collagen. However, to our knowledge there are no studies that addressed the metabolic activity of chondrocytes prepared for ACI after their expansion in monolayer culture. The purpose of our work was: 1) to analyze the anabolic response of chondrocytes prepared for autologous implantation to selected growth factors; and 2) to compare this response between two culture systems, monolayer and alginate bead. Two growth factors were chosen for this study, insulin-like growth factor-1 (IGF-1) and osteogenic protein-1 (OP-1), because they are produced endogenously by human adult articular chondrocytes and are able to stimulate the synthesis of cartilage ECM (2-4). Previously we showed that when implanted in high-density monolayer cultures (2x10^6 cell/well) or 14 days (alginate bead) with changes of media every other day. Chondrocytes were treated with OP-1, IGF-1 or a combination of OP-1 and IGF-1. Since it is critical for these cells (3,4), since in an earlier study we showed that the highest cell proliferation and PG synthesis after 48 hours of treatment, while in alginate bead cultures the effect of growth factors became apparent only by day 4; no noticeable changes were detected after 48 hours. Histological and immunohistochemical analysis of alginate beads confirmed the morphology of differentiated chondrocytes in cells prepared for ACI, and showed that by day 14 these cells produced ECM proteins specific for articular chondrocytes: type II collagen and aggrecan, although the amount of deposited proteins was relatively low. "

MATERIALS AND METHODS:
Cartilage biopsy specimens from 8 patients indicated for ACI (age 13-43 years old) were arthroscopically obtained from the affected knee. From the biopsy samples chondrocytes were isolated and cultured in monolayer for a 7-14 day period at Genzyme laboratories Genzyme Tissue Repair, Cambridge, MA). At the time of implantation, the expanded cells were returned for implantation. Remaining cell vials not used during the procedure were collected for biochemical analysis under an Institutional Review Board approved protocol. Cells were either immediately placed in high-density monolayer cultures (2x10^6 cell/well in 12-well plate) or resuspended in 1.2% sodium alginate solution at 2x10^5 cells/ml for formation of alginate beads. This cell density resulted in an average encapsulation of 20,000 cells/bead. Both cultures were maintained in the presence of 10% fetal bovine serum (FBS) for 7 (monolayer) or 14 days (alginate bead) with changes of media every other day. Chondrocytes were treated with OP-1, IGF-1 or a combination of OP-1 and IGF-1 (100ng/ml of each growth factor). In selected experiments chondrocytes in monolayers were treated with a dose of 200 ng/ml of each growth factor. Cell survival (LIVE-DEAD Cell Assay), proliferation (DNA levels), and proteoglycan (PG) synthesis (sulfate incorporation) were measured after 2, 4, and 7 days for monolayers and after 2, 4, 7, 10, and 14 days for alginate beads. Sulfate incorporation was measured over the final 4 hours of culture at each time point. After 14 days of culture, an aliquot of alginate beads was treated with a combination of OP-1 and IGF-1. Since it is critical for successful healing of cartilage defects to have not only a sufficient amount of proliferating cells, but also to have metabolically active cells that synthesize proper ECM, the results of our study suggest a therapeutic potential for combined OP-1 and IGF-1 therapy in the treatment of chondrocytes prepared for autologous implantation. However, we believe that the dedifferentiation of autologous chondrocytes immediately after seeded on the hard surface should be taken in to account when the aspect of scaffolds is considered.

REFERENCES:

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