INTRODUCTION: Autologous chondrocyte transplantation (ACT) is a possible treatment for defects in the articular cartilage of the knee. During this procedure, cartilage is harvested from an autologous donor site and isolated chondrocytes are expanded in vitro to obtain sufficient cell numbers before transplantation into the defect site. In vivo these chondrocytes are subjected to deformation due to loading resulting from normal daily activities. Physiological levels of loading cause deformations that can amount to a 22% loss of cell height. Although there is evidence that deformation has a positive effect on extracellular matrix production in normal, primary articular chondrocytes, this anabolic effect was not found in osteoarthritic (OA) chondrocytes. Moreover, during the expansion culture required for this procedure, chondrocytes tend to lose their specific phenotype. The subsequent shift towards a more fibroblast like phenotype might alter the response to deformation of these cells compared to their primary counterparts. Therefore, we investigated the effect of expansion culture of human OA chondrocytes on their response to deformation.

METHODS: Articular chondrocytes were harvested from full thickness OA cartilage of patients undergoing total knee replacement surgery. After overnight collagenase digestion, cells were counted and either seeded directly on collagen I coated 6-wells flexible plates at a density of 300,000 cells/well or plated for expansion culture in a 175 cm² culture flask at a density of 7,500 cells/cm². After three passages these cells were then seeded on collagen I coated plates at a density of 300,000 cells/well. Both the primary and expanded cells were allowed to firmly adhere to the well during a 5 day pre-culture. After the pre-culture, chondrocytes were stretched using a modified Flexcell set-up. A vacuum created under the flexible membrane of the 6-well plates pulls the membrane over a loading post, straining the adhered cells. The size of the loading post correlates to the amount of strain applied to the cells. Cells were mechanically stimulated with 0.5% or 3.0% uniaxial strain at a frequency of 0.5 Hz for two times one hour per day, with a one hour rest period in-between both cycles or left unstrained as a control. During loading, dedifferentiated cells were either cultured on basal medium (DMEM high glucose, 10% FCS) or redifferentiation medium (DMEM high glucose, 110 ITS, 25 µg/ml L-ascorbic acid, 10 ng/ml TFG-β2, 10 ng/ml IGF-1). This protocol was repeated during 3 days, after which cells were processed for RNA isolation. For every donor three wells were used per condition. Real-time RT-PCR was performed to evaluate the gene expression of matrix components aggrecan (AGC1), collagen type I (COL1), collagen type II (COL2) and proteoglycan 4 (PRG4, alias: lubrican, superficial zone protein), matrix metalloproteinases MMP1 (collagenase 1), MMP3 (stromelysin 1) and MMP13 (collagenase 3) and transcription factor SOX9. Relative gene expression was calculated according to the 2−∆∆Ct method and normalized to GAPDH. Statistical significance of loaded conditions over unstrained controls was determined using an unpaired Mann-Whitney test (P<0.05).

RESULTS: In primary OA chondrocytes, collagen type I gene expression showed a response to mechanical deformation (Figure 1, n=9, 3 donors). Collagen type I was 2 to 3 fold down regulated compared to the unstrained control. Expression of all other genes was not significantly altered by 0.5% or 3.0% strain. In dedifferentiated chondrocytes, strain drastically down regulated the gene expression of matrix proteins aggrecan, collagen type I and type II and superficial zone protein (Figure 2, n=6, 2 donors). Two of the matrix metalloproteinases responsible for degradation of the extracellular matrix were expressed higher in a response to strain. MMP1 was up regulated 30-fold and MMP3 2-3 fold, while MMP13 expression was slightly down regulated at 0.5% strain. Strain in combination with redifferentiation (chondrogenic) medium showed a similar decrease in gene expression of matrix components and an increase in gene expression of MMP1 and MMP3, although this up regulation was lower than with basal medium (data not shown, n=3, 1 donor).

DISCUSSION: In this study we investigated the difference in response to deformation of primary and culture expanded (dedifferentiated) chondrocytes. Since expansion culture of chondrocytes is an inevitable step in autologous chondrocyte replacement therapy, these dedifferentiated cells will be subjected to deformation in vivo. Our data show that these dedifferentiated chondrocytes have a more drastic response to mechanical stimulation, compared to primary chondrocytes. While gene expression levels of primary chondrocytes are hardly altered by deformation, culture expanded chondrocytes show a substantial decrease in gene expression of all tested matrix components (aggrecan, proteoglycan 4 and collagen type I and II). While the down regulation of collagen type I (~10 fold) might be favorable, the simultaneous decrease in gene expression of other matrix components, in combination with an increase in expression of the matrix degrading MMPs, MMP1 and MMP3, is highly undesirable. A chondrogenic environment does not appear to improve cellular response to deformation as all matrix molecules are still expressed in lower quantities when deformed on redifferentiation medium. In conclusion, these results indicate that deformation of culture expanded osteoarthritic chondrocytes is detrimental to the formation of a functional extracellular matrix. As a consequence, second generation ACT procedures require external factors (e.g. related to scaffolds) that optimize the chondrogenic phenotype of transplanted cells during their in vivo functioning.


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Figure 1: Gene expression of primary OA chondrocytes under strain (0.5% and 3.0%) relative to unstrained controls. * indicates statistically significantly altered expression (P<0.05). N=9, 3 donors, 3 samples per donor per condition.

Figure 2: Gene expression of expanded, dedifferentiated, OA chondrocytes under strain (0.5% and 3.0%) relative to unstrained controls. * indicates statistically significantly altered expression (P<0.05). N=6, 2 donors, 3 samples per donor per condition.