CD14/CD90 RATIO: A NEW INDEX TO MONITOR DIFFERENTIATION CHANGES IN HUMAN ARTICULAR CHONDROCYTE BASED ON CELL SURFACE MARKERS

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ABSTRACT INTRODUCTION:
To assess the efficacy of existing and novel cartilage repair techniques, reliable and reproducible outcome measurements are essential. During cell expansion in vitro, chondrocytes cultured in monolayer dedifferentiate and progressively lose their capacity to revert to the chondrogenic phenotype. This phenotype can be re-gained by different three-dimensional culturing systems in the process of re-differentiation. Current research in cartilage tissue engineering aims to optimize the re-differentiation procedures for which an accurate and sensitive assessment of the chondrocyte differentiation status is required.

To date, the assays to measure chondrocyte differentiation status have been limited to the expression of a few extracellular matrix molecules: collagen type II (COL2) and aggrecan (AGG) are the typical markers of differentiated chondrocytes, in contrast to collagen type I (COL1) and versican (VER), which are expressed on undifferentiated chondrocytes. Thus, the ratios at the mRNA level of COL2/COL1 and of AGG/VER have been used as chondrocyte differentiation indexes (1, 2). Recently, we have defined the cell surface phenotype of human articular chondrocytes (HACs), and the differential expression of certain surface markers in monolayer culture was established (3). In this study we have evaluated two cell surface markers, CD90 and CD14, for monitoring the differentiation status of HACs expanded in monolayer culture, by combining them in a novel cell surface marker-based differentiation index.

![Graph showing mRNA and Protein levels of CD14/CD90 ratio](image)

**Figure 1.** Dedifferentiation in expanded HACs based on COL2 to COL1 ratio (upper panel), AGG to VER ratio (middle panel), and CD14 to CD90 ratio (lower panel). Mean values and standard deviation are shown.

METHODS:
Cells were obtained from the lateral femoral condyles of cadaveric knee joints from six subjects (mean age 35.8 years, range 16-62) in agreement with the permission from the local ethical committee. Isolated cells were cultured in monolayer for eight passages in media containing 10% FCS. At each time point, mRNA levels of COL1, COL2, AGG, VER, CD90 and CD14 were measured by real-time RT-PCR, and protein levels of CD90 and CD14 were measured by flow cytometry. TaqMan real-time PCR was performed and monitored using the ABI Prism 7700 Sequence Detection System. Comparative Ct method was used to analyze results and 18S RNA served as internal control for cDNA input. Flow cytometry was performed on a FACScan flow cytometer, and the level of protein expression was calculated as the ratio between geometric mean fluorescence intensity of sample cells (stained with CD90 or CD14 antibodies) and that of the isotype control (3).

RESULTS SECTION:
Figure 1 shows the use of three different differentiation indexes to measure chondrocyte dedifferentiation during monolayer culture. The results reveal that dramatic changes occur as early as the first passage, confirming a recent study about rapid phenotypic changes in passaged articular chondrocytes in an animal model (4). The COL2/COL1 ratio declines steadily until passage six, and at the end of the culture (passage 8) the COL2/COL1 ratio has decreased six orders of magnitude compared to the onset of the culture. A 50-fold decrease in the AGG/VER ratio is observed at first passage, and does not further decrease until the end of the culture. A similar pattern is observed for the CD14/CD90 ratio: decrease after first passage and subsequently reach a steady level until the end of the culture. The decrease in the CD14/CD90 ratio is however higher, around three orders of magnitude, compared to the AGG/VER ratio. The protein levels of CD14 and CD90 were analyzed in parallel, and a similar trend in the CD14/CD90 ratio was observed.

DISCUSSION:
Assessment of chondrocyte phenotype is mainly performed by analysis of extracellular matrix components produced by the cell at the mRNA or the protein level. However, this approach has several drawbacks. The mRNA levels do not always correlate with the protein synthesis and post-translational modifications in the cell. On the other hand, protein detection of matrix components provides an overall result of synthesis and breakdown of these components during a period of time, without revealing the status of the cell at the time of the assessment. Our results demonstrate a correlation of CD14/CD90 ratios, at the mRNA and protein level, during HAC dedifferentiation in monolayer culture, similar to the well established differentiation index of AGG/VER (at the mRNA level only). Therefore, we propose a novel method to discriminate between differentiated and dedifferentiated HACs using a differentiation index based in the expression of CD14 and CD90. In contrast to extracellular matrix-based indexes, the cell surface marker-based differentiation index allows the determination of chondrocyte phenotype at the single-cell level by measuring the protein expression level by flow cytometry. Further validation of these surface molecules as markers for differentiation status will require to investigate whether the low CD14/CD90 ratio induced by monolayer culture can be reversed by using re-differentiation procedures.

REFERENCES:

ACKNOWLEDGEMENTS:
This work was supported by the Swiss National Science Foundation (Grants 4046-58623 and 3200B0-108371).