Isolation of Chondrocytes for Autologous Chondrocyte Transplantation Under Reduced Nutrients Stabilizes the Chondrogenic Phenotype

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Introduction: The repair of articular cartilage using autologous chondrocytes transplantation (ACT) is well established (1). For cartilage regeneration, chondrocytes have to be isolated from their surrounding matrix. In addition, cultivation and proliferation in a 2D-culture is required to enhance the cell yield.

However, articular chondrocytes have been found to dedifferentiate and lose their phenotype during isolation and culture (2,3). Because of low concentrations of glucose in cartilage (4,5), we supposed that low glucose concentration and less nutrients maintain chondrocytic properties during stressful isolation and result in a stabilized phenotype after culture.

This study was undertaken to analyze the phenotypical shift which is induced by enzymatic isolation. Here, we focused on the influence of a reduced availability of glucose. Furthermore, we will provide information of the gene profile in the subsequent 2D- and 3D-culture system.

Materials and Methods: Osteoarthritic (OA) articular cartilage was obtained from twelve patients (6 male, 6 female). Clinical data were carefully reviewed to exclude any forms of secondary OA and inflammatory joint diseases, like rheumatoid arthritis. The age of the patients ranged from 53 to 82 years (mean 75 years). Cartilage biopsies were harvested from macroscopically unaffected cartilage. One part (~ 100 mg) of the cartilage sections was frozen in liquid nitrogen and stored at -80°C as a native, untreated cartilage (NC) sample for RNA isolation. From the remaining cartilage sections chondrocytes were isolated (22 h, 37°C) with collagenase using a minimal medium (MINI, low glucose and reduced nutrients plus 5% human serum) or DMEM (plus 5% human serum), and further cultivated in DMEM. Chondrocytes of the 2D-culture were incubated for 10 d at 37°C and 5% CO2. Chondrocytes for the 3D-culture were embedded in a type I collagen gel (Arthro Kinetics) and incubated for 12 d at 37°C and 5% CO2. After isolation and after cultivation, the mRNA was isolated with TRIzol reagent (Invitrogen) according to the manufacturer’s protocol. The mRNA expression of type I, II, X collagen, and MMP-3 was quantified by qRT-PCR and normalized to GAPDH expression.

Additionally, the alkaline phosphatase (AP) activity was measured by using the BM Chemiluminescence ELISA Substrate (AP) Kit (Roche) according to manufacturer’s protocol. Glycosaminoglycans concentration was measured by using a DMB assay according to Chandrasekhar et al. (6) and normalized to DNA concentration which was quantified with Hoechst Dye 33258 (Sigma-Aldrich).

All data are expressed as means ± SEM and compared to the measurements after isolation in DMEM. Statistical significance was determined by Mann-Whitney-U-test.

Results: Already the isolation of the chondrocytes from their surrounding matrix strongly affects their gene expression. When compared to the NC, isolation in DMEM(-iso; 2.3-fold) as well as in MINI(-iso; 3.5-fold) reduced the type II collagen mRNA, whereas the type I collagen is increased in DMEM(-iso) (4.5-fold) and MINI(-iso) (5-fold). Furthermore, the isolation increased the type X collagen and MMP-3 mRNA. The gene expression of MINI(-iso) showed no significant difference compared to DMEM(-iso). However, in 2D-culture, chondrocytes isolated in MINI(-2D) showed a significant decrease in the type I/II collagen ratio (417.6-fold; p<0.05; Fig. 1). Also, the expression of type X collagen, MMP-3, as well as the AP activity was reduced. Isolation in MINI(-3D) followed by a 3D-culture, resulted in a lower type I/II collagen ratio, and reduced AP activity. In general, chondrocyte metabolism in 3D-culture was reduced.

Discussion: We could demonstrate that during isolation low glucose affects the gene expression and AP activity in 2D-cultivation. MINI-2D showed an improved Col I/II mRNA ratio, lower type X collagen, and MMP-3 expressions as well as decreased AP activity compared to DMEM-2D. These results corroborate the hypothesis of a positive influence of MINI on chondrocyte phenotype during isolation and following 2D-cultivation in DMEM. After 3D-culture, the mRNA expression was reduced generally, except the type I collagen mRNA expression which remains unchanged. The type II collagen was about 200-fold lower than the type I collagen mRNA expression after 12 d of 3D-culture. This confirms the low metabolism of chondrocytes when embedded in 3D matrices in vitro. Also Gavenis et al. observed an approximately 100-fold lower type II collagen expression after 14 d of cultivation in gel (7). In conclusion, we could demonstrate that isolation of chondrocytes using minimal medium, with low glucose, less nutrients, and supplemented with human serum, leads to chondrocytes with a less dedifferentiated phenotype. Therefore, it is attractive to speculate that these chondrocytes improve cartilage regeneration and could be a major advantage for ACT.


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