Low Concentration of Hyaluronic Acid Stabilizes the Chondrocytic Phenotype During Chondrocyte Isolation

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Introduction: Autologous chondrocyte transplantation (ACT) represents a promising solution for patients with articular cartilage defects. Chondrocytes for ACT have to be isolated by enzymatic digestion of cartilage from unloaded joint areas. Ideally, the cells obtained after isolation and culture should still have a chondrocytic phenotype. However, articular chondrocytes have been found to rapidly dedifferentiate and lose their phenotype during isolation and culture procedure. More precisely, the collagen synthesis switches from type II to type I, the synthesis of proteoglycans is reduced and the typical spheroidal cell shape changes to a flattened, fibroblast-like shape (1). Recent publications suggest that hyaluronic acid (HA) is a potent factor to maintain chondrocytic phenotype by increasing the expression of type II collagen and aggrecan (2, 3). Thus, supplementation of the isolation medium with HA could preserve the chondrocytic phenotype. Therefore, the goal was to verify this hypothesis by profiling the gene expression of chondrocytes after enzymatic digestion of articular cartilage in the presence of various HA concentrations.

Materials and Methods: Osteoarthritic (OA) articular cartilage was obtained from 6 patients undergoing total knee joint replacement. Clinical data were carefully reviewed to exclude any forms of secondary OA and inflammatory joint diseases, like rheumatoid arthritis. The age of the donors ranged from 55 to 79 years (mean 68 years). Articular cartilage was harvested from macroscopically intact areas, like rheumatoid arthritis. The age of the donors ranged from 55 to 79 years (mean 68 years). Articular cartilage was harvested from macroscopically intact areas. One part (~ 100 mg) of the cartilage sections was frozen in liquid nitrogen (mean 68 years). Articular cartilage was harvested from macroscopically intact areas, like rheumatoid arthritis. The age of the donors ranged from 55 to 79 years (mean 68 years). Articular cartilage was harvested from macroscopically intact areas. One part (~ 100 mg) of the cartilage sections was frozen in liquid nitrogen and stored at ~80°C as native cartilage (NC) samples for RNA isolation. From the remaining cartilage tissue sections, chondrocytes were isolated (22 h, 37°C) with collagenase in the presence of various concentrations of HA ranging from 0.1 to 2.0 mg/ml. Subsequently, the mRNA was isolated with TRIsol reagent (Invitrogen) according to the manufacturer’s protocol. The mRNA expression of type I, II, X collagen and MMP-3 was quantified by real time PCR and normalized to GAPDH expression. Furthermore, the alkaline phosphatase (AP) activity was measured using the BM Chemiluminescence ELISA Substrate (AP) Kit (Roche) according to the manufacturer’s protocol. All data are expressed as means ± SEM and compared to the measurements after isolation without HA. Statistical significance between means was determined by analysis of variance (ANOVA), the Kolmogorov-Smirnov test for normal distribution and two-sample t-test with heterogeneous variances (* p<0.05).

Results: Type I collagen mRNA was slightly decreased (0.77-fold) in chondrocytes isolated with 0.1 mg/ml HA. A significantly increased expression of type II collagen was detected in chondrocytes isolated with 0.1 mg/ml HA (1.95-fold; p<0.05; Fig. 1). Moreover, this type II collagen expression was 1.35-fold higher than in the NC sections. The expression of type X collagen and MMP-3 was independent of the HA concentration in the isolation medium. The AP activity was unchanged in chondrocytes isolated in the presence of 0.1 mg/ml HA compared to chondrocytes isolated without HA. Isolation of chondrocytes in the presence of higher HA concentrations led to increased AP activities.

Discussion: We could demonstrate that HA affects the expression of type I and type II collagen during chondrocyte isolation in a dose dependent manner. The most effective HA concentration was 0.1 mg/ml. At this concentration, the ratio of type II collagen to type I collagen and the alkaline phosphatase activity was most favorable. These results corroborate the hypothesis of a positive influence of HA on the chondrocyte phenotype and show for the first time that HA affects chondrocytes already during isolation. This may be due to the CD44-mediated interaction of HA with chondrocytes. CD44 is a glycoprotein expressed on the cell surface of chondrocytes which functions as a HA receptor (4). In cartilage, CD44 is involved in the modulation of the chondrocytic metabolism including internalization, cell-matrix interactions and related signal transduction (5). Therefore, the positive effect of a low HA concentration is likely caused by a negative feedback mechanism via CD44, whereby the chondrocytic phenotype is more stable at low HA concentrations. This assumption is further supported by recent publications which also demonstrate an improved chondrocyte phenotype by use of low HA concentrations in the culture medium after isolation (2, 6). In conclusion, we could demonstrate for the first time that isolation of chondrocytes in the presence of a low HA concentration stabilizes the chondrogenic phenotype. Therefore, the cultivation of these less dedifferentiated chondrocytes is likely advantageous for the following ACT.

References:

Acknowledgements: The authors thank Dr. J.A. Mollenhauer for helpful insights. This work was supported by a grant from the BMBF (0313386 A).