EFFECT OF INSULIN-LIKE GROWTH FACTOR-I ON TOTAL COLLAGEN CONTENT SYNTHESIZED BY HUMAN CHONDROCYTES CULTURED IN ALGINATE BEADS

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Introduction:
In an attempt to repair chondral lesions in a joint, different methods are under investigation that use human chondrocytes for transplantation. In our laboratory we have used the alginate system to study the effects of various growth factors and cytokines on human chondrocyte metabolism [1]. Evidence is accumulating that Insulin-like growth factor-I (IGF-I) may play a key role in maintaining the synthetic activity of adult articular cartilage [2]. Our recent studies have been focused on the potential role of exogenous IGF-I in short-term culture using alginate beads as a three-dimensional support to determine the effects of IGF-I on cell proliferation and total collagen content.

Methods:
Tissue Acquisition: Talar cartilages from the talocrural joints of 40 different donors (16-77 years of age) were obtained through the Regional Organ Bank of Illinois within 24 hours of death. The joints were graded as previously described [3] and received Collins grades 0-3 with 0 being normal with no degenerative changes, 2 with fibrillation and 3 with full thickness defects in less than 30% of the articular surface.

Cell culture: Full thickness uncalcified articular cartilage was digested, and the isolated chondrocytes were cultured at a density of 40,000 cells per bead of low viscosity alginate [1]. Human recombinant IGF-I (hrIGF-I) was re-suspended in a buffer containing 0.1% bovine serum albumin (BSA). The chondrocytes were cultured either in the absence of serum in 0.1% BSA using different IGF-I concentrations (0-100 ng/ml) or in 10% bovine serum. Media were changed and collected every second day.

Sample Preparation: For every experiment the cultures were terminated at time points 7 and 14 days. Some beads from each time point and treatment were processed for immunohistochemistry as previously described [4, 5]. The remaining beads were dissolved for 30 min in 55 mM citrate buffer at 4°C. The suspension was centrifuged to generate two fractions. The supernatant, containing the further removed matrix, and the pellet, containing the cells and their cell associated matrix, Media, cell associated matrix and further removed matrix were treated with papain overnight [1] prior to analyses for DNA and collagen.

Assay Protocols: Chondrocyte proliferation was estimated by measuring the DNA content using the bisbenzimidazole fluorescent dye (Hoechst 33258) spectrophotometrically. The number of cells per bead was estimated by conversion of DNA values, 7.8 pg DNA/cell as previously published [4]. Total collagen content was measured quantitatively with the hydroxyproline-conversion of DNA values, 7.8 pg DNA/cell as previously published [4]. DNA content using the bisbenzimidazole fluorescent dye (Hoechst 33258) was inactivated, and the digest was dialyzed against Tris buffer. Collagen in conditioned media, cell associated matrix and further removed matrix fractions was digested with pepsin overnight at 4°C; the pepsin was inactivated, and the digest was dialyzed against Tris buffer. Collagen was detectable by immunostaining in the cell associated matrix compartment after 7 days in culture. rhIGF-I at a concentration of 20 ng/ml was equivalent to 10% bovine serum in stimulating collagen synthesis above 0.1% BSA alone. Collagen was detectable by immunostaining in the cell associated matrix. By immunoblotting anti-human only type II collagen but no type I collagen alpha 2 chains could be detected. Again almost half of the type II collagen was contained in the cell associated matrix.

Conclusions:
The human adult chondrocytes in primary culture within alginate beads multiplied in the presence of the anabolic factor IGF-I under serum-free conditions. A cartilage-specific matrix with type II collagen network was detected in the cell-associated matrix compartment after 7 days in culture. These data suggest that IGF-I has a positive effect on collagen synthesis by human adult chondrocytes cultured in alginate beads and may therefore help to build up a biomechanically improved architecture in short-term culture of 7-14 days. The improved architecture should be useful for tissue engineering where there is a need to create phenotypically stable chondrocytes surround by a cartilage-specific type II collagen matrix as rapidly as possible.

References:

Acknowledgement:
Supported by NIH-grant 2-P50-AR-39239, The Dr. Scholl Foundation and Deutsche Forschungsgemeinschaft (SCHU 1267/1-1, MS, Germany). The assistance of Dr. Allan Valdellon and his staff at the Regional Organ Bank of Illinois is gratefully acknowledged.