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Effects of Hyperbaric oxygen on the expression of interleukin-1β, matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases in human osteoarthritic chondrocytes

L.J. Yuan1, S. Lin1, C. Niu1, Y. Chan1, C. Yang1, S.W.N. Ueng1, 1Chang Gung Memorial Hospital, Taoyuan, Taiwan, 2Department Of Orthopaedic Surgery And Hyperbaric Oxygen, Chang Gung Memorial Hospital, Taoyuan, Taiwan, 3Department Of Orthopaedic Surgery And Hyperbaric Oxygen Therapy Center, Chang Gung Memorial Hospital, Taoyuan, Taiwan

**Purpose:** Interleukin-1 (IL-1) plays key roles in altering cartilage matrix turnover. This turnover is regulated by matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs). Hyperbaric Oxygen (HBO) treatment improved cartilage repair. This study evaluates the effects of HBO on the expression of interleukin-1β (IL-1β), matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs) in human osteoarthritic (OA) chondrocytes.

**Methods and Materials:** Chondrocytes were released from the OA cartilage via enzyme digestion. All hyperoxic cells were exposed to 100% O2 at 2.5 atmospheres absolute (ATA) in a hyperbaric chamber. Cell growth was determined by the increase in cell numbers. The mRNA expression of IL-1β, MMPs, and TIMPs were detected by qRT-PCR. The amounts of IL-1β, MMPs and TIMPs in the conditioned medium were quantified by enzyme-linked immunosorbent assay (ELISA). Proteoglycan (PG) synthesis was determined by ex vivo incubation in N2–35%O2–6%CO2.

**Results:** Cell growth and PG synthesis increased significantly after HBO treatment. The mRNA expression of TIMP-1 increased significantly but that of IL-1β and MMP-9 decreased significantly after HBO treatment. No significant effect of HBO treatment on MMP-1, MMP-3, and MMP-13 expression. Examination of protein levels in condition medium showed that HBO treatment increased protein level of TIMP-1 but decreased that of IL-1β, MMP-1, MMP-3, MMP-9 and MMP-13.

**Conclusions:** IL-1β production in OA chondrocytes were significantly suppressed by HBO treatment, which then improved the imbalance between TIMPs and MMPs. HBO treatment may not in effect suppress suppressed by HBO treatment, which then improved the imbalance and MMP-13.

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Differentiation pattern of human normal and osteoarthritic chondrocytes

S. Concoro1, C. Brantsing2, C. Bengtsson3, C. Karlsson2, M. Brittberg4, 1Orth, Sahlgrenska University Hospital, G, Sweden, 2Molecular Cell Biology And Regenerative Medicine, Institution for laboratory and transfusion medicine, Göteborg, Sweden, 3Orthopaedic Department, Cartilage Research Unit G, Kungsbacka, Sweden, 4University, Gothenburg, Göteborg, Sweden

**Purpose:** The aim of this study was to evaluate the differentiation properties of OA chondrocytes in Hyaff 11 scaffolds and to describe the gene expression pattern in this environment.

**Methods and Materials:** Cartilage samples from 3 normal and 4 OA human patients obtained during arthroscopy or prosthesis replacement were evaluated using the Manking score in order to define the samples as normal or osteoarthritic. Chondrocytes were obtained by collagenase digestion. After the first passage cells were seeded into Hyaff 11 scaffolds at a density of 2,000 cells per cm2. For chondrocyte differentiation in 3D we used a defined culture differentiation media consisting of DMEM-HG supplemented with ITS-5.0 µg/ml linoleic acid, 1.0 mg/ml human serum albumin, 10-7 M dexamethasone, 14 µg/ml ascorbic acid and Penicillin-Streptomycin. The cells were cultured under three dimensional conditions for 21 days. Histologic sections were evaluated after Alcian blue staining. Real Time PCR was performed in order to quantify the gene expression of Collagen type I, II, Aggrecan, Versican, FGF3 and Sox 9. Different ratios were analyzed and compared.

**Results:** OA chondrocytes showed good differentiation capacity under different three dimensional conditions. Alcian blue staining was present in all the groups. The ratios between Collagen type II/ Collagen type I, Versican/Versican, FGF3 and Sox 9 were much higher than those of normal OA chondrocytes had similar differentiation capacity.

**Conclusions:** It is possible to redifferenciate OA chondrocytes under three dimensional conditions under the described conditions.

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The protective effect of OP-1 on articular cartilage in the development of osteoarthritis

R.D. Coutts1, N. Badlani1, A. Inoue2, R. Healey3, D. Amiel4; 1Orthopaedic Surgery, University of California, San Diego, La Jolla, United States of America, 2Department Of Orthopaedics, University of California, San Diego, San Diego, California, United States of America, 3Department Of Orthopaedic Surgery, University of California, San Diego, San Diego, CA, United States of America, 4Orthopaedic Surgery, University of California, San Diego, La Jolla, CA, United States of America

**Purpose:** Osteoarthritis (OA), a degenerative disorder resulting from the breakdown of articular cartilage, has many possible treatments, but none are disease modifying. Previous studies have shown that OP-1 (BMP-7) is vital to cartilage matrix integrity and repair, stimulates synthesis of cartilage matrix components, proteoglycans and collagen, and has a protective effect against catabolic mediators like MMPs and IL-1.

**Methods and Materials:** The rabbit ACLT model was used in which the ACL was transected leading to OA. OP-1 was delivered to the joint surgically by implantation of an Alzet osmotic pump into the medial thigh with a catheter threaded from the pump into the knee joint. 40 rabbits (20 control, 20 experimental underwent the ACLT surgery. They were sacrificed 9 weeks after for analysis. The OA was graded using the Outerbridge classification with India Ink staining. Semi-quantitative PCR was performed for anabolic and catabolic genes and histological staining with H&E and Safranin O was used to assess OA progression. OA progression was compared.

**Results:** The experimental group had an average Outerbridge score of 1.81 versus 2.49 for the controls (p<0.05). Semi-quantitative PCR showed a significantly greater expression of aggrecan and collagen type II in the OP-1 treated cartilage when compared to controls and less expression of another aggrecan, collagen type II. Histomorphometry showed 10.9% surface deterioration or an average loss of height of 0.05mm versus 22.3% and 0.1mm for the controls (p<0.05).

**Conclusions:** OP-1 may have a potential benefit in protecting articular cartilage during the development of OA.

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Gene expression profile of subchondral bone of osteoarthritic knee patients

T. Sasho1, R. Nagashima2, K. Nakagawa2, Y. Wada2, H. Moriya3; 1Department Of Orthopaedic Surgery, Graduate School of Medicine, Chiba, Japan, 2Orthopaedic Surgery, Graduate School of Medicine, Chiba, Japan, 3Orthopedic Surgery, Graduate School of Medicine, Chiba, University, Chiba, Japan, 4Orthopaedic Surgery, Teikyo University Chiba Medical Center, Ichihara, Japan, 5Orthopaedic Surgery, Chiba University, Chiba, Japan

**Purpose:** Subchondral bone (SB) is considered to play an important role in the development, progression, and pain generation of osteoarthritis (OA). The purpose of present study is to elucidate gene profile of osteoarthritic SB using microarray technique.

**Methods and Materials:** Three female medial-type osteoarthritic knee patients who received total arthroplasty (TKA) ages from 62-76 were involved. As an inclusion criterion, knees that had minimum OA involvement of lateral compartment were selected. SB of medial femoral condyle (MFC) that had been eburnated, and SB of lateral femoral condyle (LFC) from which cartilage were removed just after distal bone cut were frozen immediately. Total RNA were subsequently extracted and gene profile of SB of MFC and LFC were compared employing microarray (Gene Chip & reg). This chip enables us to study more than 47,000 human genes.

**Results:** About 3,000 genes were detected both in MFC and LFC. High expressions of several of these were detected and been reported such as MMP-2, 3, 9 in SB of MFC. Other than those well-known genes, what attracted us were asporin, periostin, and TRPVs (transient receptor potential) with exclusive expression in MFC in all cases. Asporin had been proved to have high association with OA susceptibility. Periostin was detected in healing process of fracture. Histomorphometry showed 10.9% surface deterioration or an average loss of height of 0.05mm versus 22.3% and 0.1mm for the controls (p<0.05).

**Conclusions:** Having selected patients with mono-compartmental osteoarthritis and considering that MFC and LFC have the same number of OA involvement of lateral compartment were selected. SB of medial femoral condyle (MFC) that had been eburnated, and SB of lateral femoral condyle (LFC) from which cartilage were removed just after distal bone cut were frozen immediately. Total RNA were subsequently extracted and gene profile of SB of MFC and LFC were compared employing microarray (Gene Chip & reg). This chip enables us to study more than 47,000 human genes.