LONG TERM FOLLOWUP OF FRESH OSTEOCHONDRAL ALLOGRAFTING OF THE FEMORAL CONDYLE

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Background and purpose: Articular cartilage injuries of the distal femur are a challenging clinical entity. Fresh osteochondral allografting (OCA) is an increasingly popular option for articular cartilage restoration. Many short-term follow up studies demonstrate promising clinical results, but there are few long-term follow up studies that provide information about graft survivorship and durability. The purpose of this study was to assess long-term clinical outcome, determine frequency and types of reoperations, determine survivorship, and to evaluate predictors of OCA failure in patients undergoing fresh OCA transplantation due to various pathologies.

Methods: Since 1983, our IRB-approved OCA outcomes program has collected data on 614 OCA transplantation procedures of the knee performed in 536 patients. Of those, we evaluated 122 patients (129 knees) who underwent OCA transplantation of the femoral condyle, who were at least ten years out from the index surgery and had a minimum two-year followup. Mean age was 31.7 years (range, 15-68 years); 85% were younger than 45 years. 53% were male 47% were female. Diagnoses included osteochondritis dissecans (45%), traumatic cartilage injury (22.5%), degenerative chondral lesion (15.5%), avascular necrosis (14.7%), and osteochondral fracture (2.3%). Average total graft area was 8.1 cm² (range, 0.7-28.5 cm²). Clinical evaluation included the modified D’Aubigne and Postel (18-point) scale, International Knee Documentation Committee (IKDC) pain and function scores, Knee Society (KS) function scores and measures of subjective satisfaction. Reoperations and failures were recorded. Graft failure was defined as revision OCA, or conversion to arthroplasty.

Results: Mean followup was 14.2 years (range, 2-27.5 years). Mean 18-point score improved from 12.1 to 16.0, mean IKDC pain score improved from 7.0 to 3.8, mean IKDC function score from 3.4 to 7.2, and mean KS-F score from 65.6 to 82.5 (all, p<0.001). Subjectively, 97% of patients were satisfied with their outcome. Forty-one knees (47%) were re-operated. Thirty-two knees (23%) had reoperations not necessarily related to the graft. Thirty-one knees (24%) failed at a mean of 7.2 ± 5.2 years (range, 1-19.7 years). Fifteen underwent revision OCA (48%) and 16 arthroplasty conversions (52%). Kaplan Meier survivorship analysis showed survival rates was 82% at ten, 74% at 15, and 66% at 20 years (figure 1). Logistic regression analysis demonstrated that age ≥ 30 years at time of surgery and having two or more previous surgeries in the operated knee were associated with allograft failure.

Conclusion: Osteochondral allografting appears useful in treating wide spectrum articular cartilage pathology. OCA resulted in durable improvement in pain and function, with graft survivorship of 82% at 10 years.

IN SITU CROSS-LINKABLE HYALURONAN FOR CARTILAGE REPAIR

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Purpose: We have investigated the feasibility of an in situ cross-linkable hyaluronan hydrogel system for cartilage repair in vitro and in vivo. The hydrogel system is a two-component system based on aldehyde-modified hyaluronic acid and hydrazide-modified polyvinyl alcohol, which are rapidly cross-linked in situ upon mixing of the two polymer components. The chemical modification of this particular hyaluronan formulation is low; approximately 5% of the carboxyl groups of the backbone are modified.

Methods: Chondrocytes or mesenchymal stem cells were encapsulated in the gel and cultured in chondrogenic medium for 28 days. The in vitro tissue formation was analyzed by histology, immunohistochemistry and dimethylmethylene blue assay for glycosaminoglycan content. The in vivo performance of the gel was studied as regeneration of local defects in a rabbit model. A defect of 4 mm diameter was created on the medial femoral condyle in the knee joints of 6 month old New Zealand White rabbits. After assuring access to stem cells from subchondral bone by bleeding, the patella was relocated in position and the knee capsule was tightly sutured before a volume of 0.4 ml of the hydrogel system was injected into the knee joint. The same defect were created on the contralateral knees, but was left untreated to serve as controls. The animals were sacrificed after 3 or 6 months and the joints processed for histopathological examination.

Results: We demonstrated that chondrocytes and mesenchymal cells cultured in the hydrogel form cartilage-like tissue, rich in glucosaminoglycans, collagen type II and aggrecan. In the rabbit animal model the injection of the hydrogel improved the healing of full-thickness cartilage defect in the knee as compared to non-treated controls. The in vivo study showed that the regenerated cartilage defects stained more intense for type II collagen upon treatment with the hydrogel. Here and in previous studies of bone regeneration using these materials, no inflammatory response or capsule formation has been observed.

Conclusions: The results are very promising, and the hydrogel system could be used both for cell based and cell free regeneration therapies. Future studies are aimed to explore and develop a new way to contribute to the repair of impaired bone and cartilage tissue in osteoarthritis. This is based on the known facts that inflammatory stimuli as well as mechanical load contributes to cartilage and bone damage in OA, and that repair demands that the tissue should be supported with 1) adequate biomechanical stimuli 2) suppress destructive inflammation and 3) stimulate regeneration with anabolic factors. The hyaluronan hydrogel system would provide the biomechanical support and ability to deliver anti-inflammatory and anabolic factors to promote regeneration.

THE CHONDROPROTECTIVE EFFECT OF FGF9 IN AN EXPERIMENTAL MODEL OF OSTEOARTHRITIS

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Purpose: To study the chondroprotective effect of local delivery of FGF9 in a rat meniscal tear model of osteoarthritis.

Methods: 34 skeletally mature male Lewis rats underwent surgery to create full-thickness transection of the medial collateral ligament and the medial meniscus of the right knee. Two weeks after surgery animals were randomized into two treatment groups: 18 animals received bi-weekly intra-articular injections of 4 μg FGF9 in 50 μl saline for total of 3 weeks. A parallel treatment group included 16 animals that received only 50 μl saline. Three days after the last injection, animals were euthanized and 40 joints (6 normal, 16 surgery, 18 surgery + FGF9) were processed and sections stained with toluidine blue. Quantitative and semi-quantitative histopathological evaluation was performed to assess the osteoarthritic changes of the operated joints and the effect of FGF9 treatment in the medial tibial plateau region. The parameters that were evaluated include: cartilage degeneration score, tibial cartilage degeneration width, cartilage thickness and depth of lesions, semi-quantitative collagen damage assessment, percent proteoglycan loss, and cartilage viability.
**Results:** FGF9 had significant beneficial effects on multiple parameters used to assess cartilage damage. FGF9 treatment reduced the cartilage degeneration score for the outer region of the medial tibial plateau by 33% (p = 0.004), the width of significant cartilage damage by 38% (p = 0.018), and the depth of cartilage lesions by 20-44% (p = 0.003). Image analysis showed that FGF9 increased the total cartilage area by 24% (p < 0.001) and the viable cartilage area by 35% (p < 0.001). Proteoglycan loss was reduced by 43% (p = 0.003) and the area that showed minimal damage to the collagen was increased two-fold. FGF9 had no significant effect on the subchondral bone but increased the size of the chondrocytes/osteophytes by 29% (p = 0.018), and the depth of cartilage lesions by 20-44% (p = 0.0014) significantly increased in OA cartilage compared to normal cartilage. When we analyzed regions (BSQ1-5) from −4548 to −2846 in the promotor of SOX-9 by bisulfite sequencing, methylated CpG sites significantly increased in all the examined regions; total methylated CpG sites increased about eight-fold in OA cartilage (14.04%) than in normal cartilage (1.66%).

**Conclusions:** Our study suggests that the increased methylation status in the SOX-9 promoter region may have a close relation to the progression of OA.

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**65 MICROARRAY STUDIES OF SYNOVIAL SPECIMEN OF EARLY HUMAN (CHECK) AND EXPERIMENTAL OA IDENTIFY PATHWAYS AND PROCESSES ASSOCIATED WITH CARTILAGE DAMAGE**

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**Purpose:** Over 50% of osteoarthritis (OA) patients show synovial inflammation, even relatively early during the disease. However, the mechanisms through which this synovial activation contributes to the irreversible joint pathology that characterizes OA, are not known. In the present study we used microarray analysis of synovial tissue of early OA patients and of experimental OA, to identify common pathways that determine cartilage damage in this disease.

**Methods:** From a subpopulation of patients that entered the CHECK Cohort study (Cohort Hip and Cohort Knee), synovial biopsies were collected. CHECK is a prospective 10-year follow-up study that was initiated by the Dutch Arthritis Association on participants with early osteoarthritis-related complaints of hip and/or knee. Radiographs are taken in a standardized manner and scored (Kellgren-Lawrence KL) at inclusion (n=18). In addition, biopsies of 7 control synovia were collected. A longitudinal expression analysis was performed on murine synovial tissue at day 7, day 21 and day 42 after induction of collagenase induced OA (CIOA). CIOA was induced by intra-articular injection of collagenase, which causes joint instability, and contra lateral knee joints served as controls. Initial analysis of microarray data was performed using Partek software and functional annotation clustering (FAC) and pathway analysis was done using DAVID.

**Results:** Gene expression profiles of control synovia were compared to CHECK synovia. Analysis using DAVID indicated enrichment of several biological processes and signaling pathways, including regulation of macrophage differentiation, innate immune responses, cell migration, TGFβ-, BMP- and wnt-signaling. This indicates clear activation of the synovium in the CHECK patients compared to controls. Next we compared synovial tissue of CHECK-patients with radiological damage (KL≥1) with CHECK-patients without joint damage (KL<0). Among the top 30 genes that were strongest associated with cartilage damage were MMP-1 (18-fold), MMP-3 (10-fold), S100A8 (6-fold) and cartilage glycoprotein-39 (6-fold), all of which have been associated with cartilage damage. Immunohistochemical staining revealed that expression of MMP-1 and MMP-3 was highest in the synovial lining layer. FAC analysis showed that, among others, response to wounding, chemotaxis, innate immune response and metalloproteases were strongly and significantly enriched and thus associated with joint damage. Pathway analysis demonstrated that in the synovium of patients with joint damage the complement-activation pathway, TGFβ- and BMP-signaling and TLR-activation were significantly upregulated. These results were further underlined by analysis of synovium from experimental OA. Among the genes that were strongly upregulated on all 3 time points after induction were MMP-3 (6-fold), MMP-13 (16-fold), MMP-14 (6-fold) and COMP (13-fold). Again, wound healing, innate immune response and metalloproteases were significantly enriched, as were the complement pathway, the TLR-, TGFβ, BMP and wnt-signaling pathways. In a recent publication, complement was demonstrated to be essential in experimental OA. We therefore determined whether