9.1.1 Pi88 is effective in Reducing Cartilage Degeneration and Promoting Cell Survival in the Model of Acute Trauma to Human Ankle Cartilage: the Mechanism of Action

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Introduction: Objectives: To investigate the effect and understand the mechanism of action of Pi88 surfactant on cartilage degeneration and cell survival in acute trauma to human ankle cartilage.

Methods and Materials: Sixteen normal tali were impacted using a 4mm indenter with 600N. 8mm cartilage plugs containing the 4mm impacted core and 4mm immediately adjacent non-impacted ring were removed and cultured with or without Pi88. Results were assessed by layers at 0.27 and 14 days after injury with live/dead, Tunel assays and histology with Safranin-O/fast green staining. Pi88 mode of action was assessed via its regulations of IL-6 and MAPK signaling using Western blots.

Results: A single impact to human articular cartilage resulted in cell death at the impaction site and radial progression of apoptosis to adjacent ring. Pi88 promoted cell survival by reducing cell death more than 2-fold (p<0.05) in the core and about 30% in the ring as compared to all untreated controls. It also inhibited expansion of apoptosis in the ring especially within first 7 days post impaction (7.5% Tunel-positive cells while with 10ng/ml TGF-β1 46% in the un-impacted control (p<0.05)). In the current study Pi88 mediated its effect through the inhibition of phosphorylation of MAPK/ERK, JNK, and p38 and attenuation of IL-6 signaling via inhibition of Stat1 and Stat3; furthermore, phosphorylation of ATF2 (downstream of p38 pathway) was also affected.

Conclusions: Observed results and identified novel mechanisms of Pi88 action suggest its therapeutic potential alone or combined with anabolic agents in preventing progressive cartilage degeneration and the development of post-traumatic OA.

9.1.2 Mechanically induced Chondrogenesis of Human Bone Marrow Mesenchymal Stem Cells is regulated via TGF-β

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Introduction: Mesenchymal stem cells derived from bone marrow (MSCs) have the potential to differentiate into chondrocytes. This study investigated the effect of dynamic compression and surface rotation on hMSC chondrogenesis. We also investigated the influence of transforming growth factor β1 (TGF-β1) on the response of hMSCs to load.

Methods and Materials: Human MSCs (5×106) were seeded into fibrin-polyurethane scaffolds. Scaffolds were pre-cultured for 7 days in ITS+DMEM containing either 0, 1, or 10ng/ml TGF-β1, then loaded 1 hour daily for 7 days. Measurements included DNA, glycosaminoglycan and mRNA expression of collagen type I, II, and X, aggrecan, TGFβ1, TGFβ3, proteoglycan-4, and Sp7. TGF-β1 protein was measured by ELISA. TGF-β1 signaling was blocked using the TGF-β receptor-I selective inhibitor Ly364947.

Results: Load stimulated GAG synthesis and significantly increased the expression of all chondrogenic markers in the absence of added TGF-β1. The effect on gene expression was far smaller when 10ng/ml TGF-β1 was added and intermediate with 1 ng/ml. Without added TGF-β1, load significantly up-regulated TGF-β1 protein synthesis. While load significantly up-regulated TGF-β1 protein synthesis by 20%. Addition of Ly364947 significantly reduced the chondrogenic gene expression compared with vehicle control.

Conclusions: Different TGF-β concentrations influenced the effect of mechanical load on the chondrogenesis of hMSCs, with the greatest effects being seen at lower TGF-β concentrations. Under lower concentrations of TGF-β1, load up-regulated TGF-β1 gene expression and TGF-β1 protein synthesis. Blocking of TGF-β receptor-I signaling abrogated this response. This indicates the mechanically induced chondrogenesis of hMSCs is an outcome of increased TGF-β synthesis and signaling.

9.1.3 The Response of Articular Chondrocytes from Different Topographical Locations of the Knee to Mechanical Stimulation in vitro

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Introduction: Biopsies for chondrocyte transplantation are commonly obtained from the femoral notch irrespective of the desired site of re-transplantation. We hypothesize that cells, isolated from different topographical locations of the knee, display an unequal matrix/gene expression behavior when subjected to simulated in vivo conditions.

Methods and Materials: Chondrocytes from eight different topographical locations of bovine stifle joints, weight-bearing: medial/lateral femoral condyle and tibia, patella/trochlea and non-weight-bearing: femoral notch and proximo-medial femoral condyle, were seeded at passage 3 within 3-D scaffolds to be equally divided into mechanically-loaded (custom-designed bioreactor) and unloaded (control-free swelling) groups. Constructs were analysed for DNA, scaffold, GAG/scaffold and Collagen-I,-II,-X, COMP, AggreCan, Sox9, PRG-4, PTHrP and MMP-1, -3,-13 mRNA after 16 days of culture.

Results: No difference in DNA content was found under any condition, while significant differences in GAG content between controls were found among control and loaded groups, respectively. Between control groups, mRNA expression was different for all genes except for Sox9, PRG-4 and PTHrP. This difference remained among loaded groups where only AggreCan, Sox9 and PTHrP were similar. In comparison to control, GAG and all genes were significantly higher in response to load except for Collagen-I,-X, MMP-1 and-3.

Conclusions: Our results demonstrate that articular chondrocytes from different topographical locations of the knee joint behave differently under free swelling conditions and this is more pronounced under mechanical conditions. This unequal behavior should be taken into consideration when performing autologous chondrocyte transplantation to improve cell-based cartilage repair techniques to the maximum tissue performance and possibly clinical outcome in the future.

9.1.4 Effects of compression on human subchondral osteoblast metabolism

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Introduction: Recent data showed that subchondral bone plays an important role in osteoarthritis (OA). Metabolic and morphologic modifications in this tissue contribute to the degradation of the overlying cartilage. It was suggested that abnormal mechanical pressure exerted onto the articulation was responsible to these changes. Here, we evaluated the effects of compression on osteoblasts from subchondral bone.

Methods and Materials: Osteoblasts were isolated from sclerotic (SC) or non-sclerotic (NSC) areas of human OA subchondral bone. After 28 days, osteoblasts were surrounded by their matrix. This osteoblasts-containing membrane was then placed onto a Biopress Flexercell plate and submitted to a 4h 1.67 MPa compression (1 Hz). Expression of IL-6, IL-8, COX-2, VEGF, IGF-1, OPG and RANKL was evaluated by RT-PCR. IL-6, IL-8 and PGE2 were quantified by ELISA.

Results: Basal IL-6, VEGF, COX-2, IGF-1 and RANKL mRNA levels were significantly increased in SC osteoblasts as compared to NSC. By contrast, SC osteoblasts expressed less OPG than those from NSC areas. Compressions induced the expression of genes coding for IL-6, IL-8, COX-2, IGF-1, VEGF and RANKL but decreased the expression of OPs in NSC osteoblasts (p<0.05). Interestingly, compressed NSC osteoblasts expressed similar levels of these genes than SC osteoblasts.

Conclusions: We show that our model of compression can induce in NSC osteoblasts a phenotype similar to this observed in sclerotic areas. Moreover, SC osteoblasts are less sensitive to mechanical stimuli than NSC osteoblasts. These results clarify the role of compression in the pathogenesis of subchondral bone sclerosis and allow new perspectives of research in this field.
9.2.5 In vivo small animal model to study cartilage repair
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Introduction: Subcutaneous or intramuscular implantation of different cell-polymer or biomaterial constructs in athymic (nude) rodents is frequently used to screen materials for cartilage repair. However, unlike in the articular joint, the environment surrounding implant in rodents is highly vascular, normoxic and without cartilage cytokines. We addressed this inadequacy by creating a local “chondrogenic environment” in the intramuscular site in athymic rats. We performed an initial proof of concept study to validate ability of such “chondrogenic environment” to preferentially stimulate formation of cartilage. Demineralized bone which typically forms bone in intramuscular environment of rat was implanted in the devised “chondrogenic environment” and evaluated for cartilage vs. bone formation.

Methods and Materials: A “chondrogenic environment” is created by gluing two pieces of viable human cartilage to form a cartilage cap. This cap was filled with human demineralized bone and implanted in intramuscular pouches of 24 athymic rats for 4 weeks. Demineralized bone without cartilage cap has served as control. The explants were stained with H&E, Safranin O and for collagen II and amount of newly formed cartilage and bone evaluated histomorphometrically.

Results: After 2 weeks, both implanted DBM control and DBM in cartilage cap showed similar quality of formed cartilage that appeared to be mature and hyaline. After 4 weeks, DBM control continued along endochondral bone formation while DBM in cartilage cap showed 3 fold larger amount of cartilage than control, and almost no bone formation (p<0.05).

Conclusions: Our results show that the assembled cartilage cap can provide “chondrogenic environment” that, in turn, can increase cartilage and slow down bone formation.

9.1.6 Stimulation of Engineered Cartilage Constructs by Sliding Motion Results in Improved Micro- and Nanometer Scale Surface Properties
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Introduction: Functional tissue engineering aims to generate cartilage-like constructs with matrix and surface properties standing high forces in joint motion. In this study a bioreactor system that simulates natural joint movements was employed to mechanically stimulate cell-scaffold constructs.

Methods and Materials: Polyurethane scaffolds were seeded with bovine chondrocytes and subjected to dynamic compression, applied by a ceramic ball that was pressed onto the scaffold, for 1h daily (Group 1). In Group 2, the ball additionally oscillated over the scaffold, generating sliding surface motion. After 3 weeks, the surface quality of the engineered constructs was analyzed by atomic force microscopy, i.e. by imaging (including surface roughness measurements), by measuring their micro- and nanostiffness based on indentation testing, and by measuring surface friction forces. The results were compared and correlated to biochemical, histological, and immunohistochemical analyses.

Results: Micro- and nanostiffness were highest in Group 2, followed by Group 1 and control groups. Interestingly, also collagen II staining was most intense in Group 2. Safranin O staining was similar in Groups 1 and 2 but higher compared to the unloaded controls, demonstrating increased accumulation of proteoglycans in loaded specimens. Group 2 also exhibited lowest surface roughness and friction values that correlated with more pronounced elastic and frictional properties.

Conclusions: Here we demonstrate that stimulation by sliding motion resulted in the most improved cartilage surface properties. Moreover, the ability to directly relate structure/function relationships of de novo generated cartilaginous constructs at various scales critical to function is of potentially great value. This may open an approach to produce engineered cartilage similar to normal cartilage.

9.1.7 Functional tissue engineering of articular cartilage using adult chondrocytes
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Introduction: The objective of this study was to determine the efficacy of a functional tissue engineering approach for cartilage tissue engineering using adult chondrocyte-seeded hydrogel constructs and applied dynamic deformational loading.

Methods and Materials: Adult canine chondrocytes were passaged up to two times in DMEM with 5% FBS, TGF-beta3, FGF-2, and PDGF-BB. Chondrocytes were suspended in 2% agarose at 30 million cells/mL. Discs (d=4.0 x t=2.4mm) were cultured in serum-free chondrogenic media supplemented with TGF-beta3 (10 ng/mL) for the culture duration. Engineered constructs were subjected to free-swelling (FS) or dynamic loading (DL: 10% deformation, 1Hz, 3 hours daily, starting culture day 28).

Results: Chondral constructs exhibited a significant ~50% increase in Youngs modulus (Ey) with applied dynamic loading. The GAG and collagen content were similar for FS and DL groups, ~5%ww and 8%ww respectively. Aggrecan and type II collagen expression were doubled with loading. For DL constructs (day 63), Ey=350 +/- 33 kPa (n=4). Properties achieved in this study exceed native canine groove cartilage properties (Ey=201 +/- 88 KPa, n=5 dogs) and are comparable to medial condyle cartilage.

Conclusions: Our findings successfully demonstrate a functional tissue engineering strategy incorporating clinically-relevant, adult chondrocytes for engineering cartilage replacement tissue. These results are in contrast to our previously reported studies with immature chondrocytes where the sequential application of dynamic loading after transient TGF-beta3 application was found to be the optimal protocol. Prerequisite studies using adult allogeneic chondrocyte-seeded constructs (free-swelling) with native properties implanted into cylindrical defects in the patellar groove show promising tissue repair.

9.1.8 The vimentin cytoskeleton contributes to chondrocyte stiffness and changes with OA
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Introduction: The chondrocyte cytoskeleton contains actin microfilaments, tubulin microtubules, and vimentin intermediate filaments. In chondrocytes, actin and tubulin are studied extensively but less is known about vimentin. In other cells, vimentin affects cell stiffness, and acrylamide disrupts vimentin’s role in cell structural integrity. Here we determined vimentin’s contribution to cell stiffness using 3D-cultures of primary human chondrocytes from normal and osteoarthritic cartilage.

Methods and Materials: Chondrocytes were embedded into 2% alginate discs and cultured 24hrs +/- 4mM acrylamide to disrupt vimentin organization. Compression of 20% bulk strain was applied using a custom device on the stage of a confocal microscope. Deformation was measured as the ratio of the X:Y diameters of ~1900 cells from 9 donors. We used 3D-cultured passaged human chondrocytes expressing GFP-vimentin to visualize vimentin after acrylamide treatment and compression. For each X:Y ratio, cellular stiffness was estimated by Finite Element Modeling alginate-embodied chondrocytes under 20% strain.

Results: Using a 3D culturing system, vimentin formed a dense cage-like structure well within the cortical actin shell. Untreated normal cells were the stiffest, and disruption of vimentin reduced the measured cell stiffness significantly (~2.8-fold according to FEM estimations). OA chondrocytes were not as stiff initially and less affected by vimentin disruption, consistent with observations that arthritic chondrocytes have abnormal vimentin organization. During compression, the GFP-vimentin “cage” deformed less than the cytosol, an effect no longer seen after vimentin disruption.

Conclusions: Examining cell stiffness in matrix-embedded chondrocytes may reveal new roles for vimentin’s role in cell stiffness, protection from mechanical injury, and arthritis progression.
9.1.9 Cartilage regeneration in a scaffold-free diffusion-culture
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**Introduction:** Matrix assisted ACI is used nowadays with varying results. As alternative, scaffold free repair techniques are discussed. Aim of this study was to examine a scaffold-free diffusion-culture model, which uses mega-congregates of chondrocytes cultured at an air-medium interface. This scaffold-free diffusion-culture (SFDC) could be used to repair cartilage defects.

**Methods and Materials:** Human chondrocytes (P1-7) were expanded in monolayer and transferred to pellet-culture or SFDC. After one week, cultures were stained with alcian blue and safranin-O and evaluated by immunohistochemical staining for type II collagen. Quantitative real time reverse transcriptase polymerase chain reaction (qRT-PCR) was performed for mRNAs of cartilage markers.

**Results:** Positive alcian blue staining was detectable in SFDC up to P7. There was a positive signal for collagen type II in SFDC up to P7. In qRT-PCR a redifferentiation of human chondrocytes was shown by the transfer into SFDC. Within P1 to P3 human chondrocytes which were cultured in monolayer lost the ability to express collagen type II but could regain it if they were transferred to SFDC. At SFDC chondrocytes showed the highest expression of collagen type II at passage 1 when compared to monolayer or to pellet-culture.

**Conclusions:** It could be shown that the cultivation in SFDC can lead to redifferentiation of human chondrocytes. Chondrocytes in SFDC tend to form their own matrix and produce collagen type II at higher amounts than in monolayer or in pellet-cultures. Therefore diffusion-culture congregates might be an appropriate tool to be used for a new scaffold-free cartilage regeneration approach.

9.2.1 Human Embryonic Stem Cell-derived Mesenchymal Stem Cells Promote Cartilage Repair by Paracrine Action
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**Introduction:** ESCs are pluripotential, but their application for cartilage regeneration has not been explored. Our study was designed to explore the impact and underlying mechanism of human embryonic stem cell-derived mesenchymal stem cells (hESC-MSCs) on improving the therapeutic efficacy of cartilage defect repair.

**Methods and Materials:** Full-thickness cartilage defects (diameter=2 mm, thickness=2 mm) were made in the patellar grooves of male SD rat and randomly divided into three groups: control group, collagen scaffold group, DiI labeled hESC-MSCs in collagen scaffold group. The University of Zhejiang Institutional Animal Care and Use Committee approved this research protocol.

**Results:** hESC-MSCs were demonstrated to exist in defect areas at least for 4 weeks as evidenced by the detection of Dil fluorescence and human b-actin mRNA expression. The histological results showed that the group treated with hESC-MSCs had more amounts of hyaline cartilage, more glycosaminoglycans content, higher ICRS histological scores and Young’s modulus of the repaired cartilage tissue at both 4 and 8 weeks. Moreover, the mRNA expression levels of human TGF-b3, collagen type II, aggrecan and SOX9 were significantly increased in hESC-MSCs after they were implanted into the defect area.

**Conclusions:** We observed that hESC-MSCs were demonstrated to exist in defect areas at least for 4 weeks as evidenced by the detection of Dil fluorescence and human b-actin mRNA expression. The histological results showed that the group treated with hESC-MSCs had more amounts of hyaline cartilage, more glycosaminoglycans content, higher ICRS histological scores and Young’s modulus of the repaired cartilage tissue at both 4 and 8 weeks. Moreover, the mRNA expression levels of human TGF-b3, collagen type II, aggrecan and SOX9 were significantly increased in hESC-MSCs after they were implanted into the defect area.

**Introduction:** Our results demonstrated that hESC-MSCs can improve the regeneration of cartilage tissue by promoting TGF-b3 secretion and paracrine action and induce chondrogenic differentiation of human embryonic stem cells (hESC-MSCs) on cartilage repair and promote cartilage repair by paracrine action.

9.2.2 Characterization of tet-regulated, lentivirally mediated BMP-2 expression in primary rabbit chondrocytes for cartilage regeneration
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**Introduction:** Therapy of cartilage defects is still challenging due to poor self-healing-capacity. A combined gene- and cell-therapeutic approach for in-situ production of growth factor BMP-2 by implanted autologous chondrocytes might be advantageous. To allow for regulation of gene-expression the tet-on-system was chosen, delivered by VSV-G-pseudotyped lentiviral-vectors.

**Methods and Materials:** Experiments were carried out on primary rabbit chondrocytes. Cells were coinfected with 2 vectors, expressing rtTA and eGFP or BMP-2 under control of TRE, respectively. Transgene-expression was induced by doxycycline (dox). Transactivator vectors with different promoters driving rtTA-expression were compared. Additionally a 1-vector-system for expression of the transactivator and eGFP or BMP-2 using the strongest promoter was constructed. Results were obtained by FACS-analysis for eGFP-expression or ELISA for secreted BMP-2. Synthesis of proteoglycans was determined by alcian blue staining.

**Results:** Efficiency of tet-on-system was tested using eGFP. Dox-induction was weakest in 2-vector-system with CMV-driven rtTA-expression and coinfection with TREeGFP(4.4x). When the rtTA was driven by SF- or PGK-promoter eGFP-expression was comparable(45.5/68.4x). Inducibility was also comparable with a 1-vector-system with SF-driven rtTA(44.3x). The overall expression was 2x higher with the 1-vector-system. The same setup using BMP-2 resulted in production of approx. 16ng/ml BMP-2 in both systems. BMP-2 was not detected in the absence of dox. Maximal-induction of BMP-2-expression was found after 5 days and repeated addition of dox. After dox-removal expression returned to baseline. BMP-2 was functional for proteoglycan-synthesis.

**Conclusions:** The tet-on-system allows regulation of BMP-2-expression. BMP-2-production by chondrocytes after induction is sufficient for proteoglycan-synthesis. The 1-vector-system is favorable over the 2-vector-system since it allows reduction of virus-load and thus increasing vector-safety.

9.2.3 Extent of hypertrophy induced by BMP-2 and BMP-4 gene transfer in different tissue segments of cartilage: human MSCs
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**Introduction:** Hypertrophic differentiation of transplanted chondrocytic cells is thought to limit cell-based cartilage repair, as it leads to apoptosis and ossification. The present study compares BMP-2/-4 gene transfer as agents of chondrogenesis and hypertrophy in human MSC pellet cultures.

**Methods and Materials:** Cultures of marrow MSCs were infected with 5 x 102 particles/cell of Ad.BMP-2 or Ad.BMP-4, seeded into 12-well plates and cultured for 3 weeks in serum-free medium. Untransduced or marker gene transduced cultures served as controls. Expression of BMP-2 and BMP-4 was determined by ELISA, and aggregates were analyzed histologically, immunohistochemically, biochemically and by RT-PCR for chondrogenesis and hypertrophy.

**Results:** Levels of BMP-2 or -4 in the media were initially 35-55 ng/mL and declined thereafter. BMP-2 and BMP-4 genes were equipotent inducers of chondrogenesis in primary MSCs as judged by lacuna formation. Young’s modulus of the repaired cartilage tissue at both 4 and 8 weeks of the group treated with hESC-MSCs had more amounts of hyaline cartilage, more glycosaminoglycans content, higher ICRS histological scores and Young’s modulus of the repaired cartilage tissue at both 4 and 8 weeks. Moreover, the mRNA expression levels of human TGF-b3, collagen type II, aggrecan and SOX9 were significantly increased in hESC-MSCs after they were implanted into the defect area.

**Conclusions:** We observed that hESC-MSCs were demonstrated to exist in defect areas at least for 4 weeks as evidenced by the detection of Dil fluorescence and human b-actin mRNA expression. The histological results showed that the group treated with hESC-MSCs had more amounts of hyaline cartilage, more glycosaminoglycans content, higher ICRS histological scores and Young’s modulus of the repaired cartilage tissue at both 4 and 8 weeks. Moreover, the mRNA expression levels of human TGF-b3, collagen type II, aggrecan and SOX9 were significantly increased in hESC-MSCs after they were implanted into the defect area.

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**Results:** Levels of BMP-2 or -4 in the media were initially 35-55 ng/mL and declined thereafter. BMP-2 and BMP-4 genes were equipotent inducers of chondrogenesis in primary MSCs as judged by lacuna formation.
formation, strong staining for proteoglycans and COL II, increased levels of GAG synthesis, and expression of mRNAs associated with the chondrocyte phenotype. However, BMP-4 modified aggregates showed a lower tendency to progress towards hypertrophy, as judged by expression of alkaline phosphatase, immunohistochemical staining for type X collagen protein, and lacunae size. **Conclusions:** BMP-2 and -4 were equally effective in provoking chondrogenesis by human MSCs in pellet culture. However, chondrogenesis triggered by BMP-4 gene transfer showed less evidence of hypertrophic differentiation than that triggered by the BMP-2 cDNA. In the latter case, the cells resembled growth plate chondrocytes both morphologically and functionally. This suggests that BMP-4 may be a better candidate than BMP-2 for use in cartilage repair.

**9.2.4 Chondroprotection of rat articular chondrocytes by ADAMTS-5 antisense sequence and gene delivery by AAV**

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**Introduction:** Aggrecan degradation is an early feature of both osteoarthritis and inflammatory arthritis. ADAMTS-5 (TS-5) is a major aggrecanase in articular cartilage with protection against cartilage erosion and arthritis occurrence in TS-5 KO mice. This study tested the hypothesis that TS-5 antisense gene therapy will have chondroprotective effects, and that it can be delivered to chondrocytes using adeno-associated virus (AAV).

**Methods and Materials:** Primary rat articular chondrocytes were transfected with a plasmid encoding antisense TS-5 sequence, followed by an inflammatory stimulus, such as IL-1beta for 24 hours. TS-5 gene expression was determined by qPCR. Rat femoral explants were divided into three groups for transduction with AAV-Luciferase (AAV-Luc): Control (no AAV), AAV-Luc, and mechanical damage via scratch before AAV-Luc. All explants were imaged for luciferase 96 hours post-infection.

**Results:** TS-5 gene expression was reduced above 80% with TS-5 antisense plasmid, IL-1beta stimulation amplified TS-5 expression, and its effect was abolished with TS-5 antisense transfection. Luciferase expression was observed in femoral explants, with a brighter bioluminescence in condyles that were scratched before AAV infection.

**Conclusions:** This study showed that this novel antisense sequence targeting TS-5 mRNA suppresses TS-5 gene expression. The abolishment of IL-1beta induced TS-5 overexpression suggests that the antisense treatment may protect cartilage in inflammatory conditions. The ability of AAV to transduce chondrocytes within articular cartilage, and its increased efficiency at damaged areas has additional therapeutic implications for targeted gene therapy following mechanical injury. This study supports the potential delivery of TS-5 antisense to articular chondrocytes by AAV gene transfer as a chondroprotective strategy.
9.2.7 Treatment of Full Thickness Chondral Defects with a Collagen Scaffold, Mesenchimal Stem Cells Compromised to the Chondrocyte Lineage and Platelet Rich Plasma. A. Vaisman1, D. Figueroa1, R. Calvo1, M. Espinoza2, M. Gallegos1, P. Conget1.
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Introduction: Purpose: To evaluate the macroscopy, histology and molecular properties of the repair tissue generated after treating acute full thickness chondral defects of the knee with a bi-layer collagen scaffold (BSC) embedded with mesenchimal stem cells compromised to the chondrocyte lineage (MSC) and platelet rich plasma (PRP), in a rabbit model.

Methods and Materials: Experimental study in New Zealand adult male rabbits. 20mm2, acute full thickness chondral defects were surgically induced in 36 femoral condyles. Each rabbit was randomly included into one of four groups: 1.- Group 1 (8 femoral condyles): chondral lesion left untreated (control group). 2.- Group 2 (12 femoral condyles): scaffold without cells nor PRP. 3.- Group 3 (8 femoral condyles): BSC + MSC. 4.- Group 4 (8 femoral condyles): BSC + MSC + PRP. 6 months after surgery the repair tissue was studied macroscopically, histologically (H-E and Toluidine Blue) and molecularly (qRT-PCR of Collagen/ Collagen and agrecan/versican). Statistical analysis was performed with ANOVA test (p<0.05).

Results: a) Macroscopy: group 4 exhibited properties similar to normal hyaline cartilage. All the other groups had a fibrocartilage type of repair. b) Histology: all the groups had at least some presence of fibrocartilage, with no differences among groups. c) Molecular analysis: Groups 3 and 4 had a significantly higher relation between col2/col1 than groups 1 and 2 (p<0.0001).

Conclusions: Treatment of full thickness chondral defects with a collagen scaffold, mesenchimal stem cells compromised to the chondrocyte lineage and platelet rich plasma shows promising results. Nevertheless, none of the treatment groups healed with normal hyaline cartilage.

9.2.8 Transplantation of TGF-β1-Rejuvenated Periosteum for Osteochondral Tissue Regeneration. G.G. Reinholz1, J.S. Fitzsimmons, A.O. Meza, M.E. Casper, T.J. Ruesink, S.W. O’Driscoll.
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Introduction: Previously we demonstrated that local injection of TGF-β1 increases cartilage cell number and in vitro chondrogenesis in aged periosteum. Subsequently, we examined the efficacy of TGF-β1-rejuvenated periosteum to regenerate osteochondral tissue in vivo.

Methods and Materials: Twelve-month old rabbits received four subperiosteal injections (evenly spaced along the graft harvest site) of TGF-β1 (200 ng) percutaneously (7 days pre-op only) or no injection. A 5 mm wide osteochondral defect (spanning the width of the patellar groove) was created in one limb/rabbit. A TGF-β1-injected or non-injected periosteal graft was then sutured to the base of the defect with the cambium layer up. After surgery rabbits were allowed unrestricted motion.

Results: At six weeks post-op, the defects were completely filled with regenerated tissue in both the TGF-β1-injected and non-injected groups with integration into the surrounding tissue and reformation of the original contours of the patellar groove. The histological score for the regenerated tissue (max=30) in the TGF-β1-injected group was significantly higher (p<0.05) than the non-injected group (14.4 ±2.1, n=7). The histological scores for the contralateral limbs from both the TGF-β1-injected (29.8 ±0.3, n=8) and non-injected (29.9 ±0.2, n=7) rabbits were significantly higher than the surgical defects (p<0.0001). No significant differences in equilibrium modulus or GAG content were found between any of the groups, while percent type II collagen was significantly higher in the contralateral limbs compared to the surgical limbs (p<0.05).

Conclusions: These results demonstrate that TGF-β1-rejuvenated periosteum restores improved osteochondral defect repair at six weeks post-op compared to untreated periosteum.

9.2.9 Reduced hypertrophy and enhanced stabilization of the chondrogenic phenotype in MSC by co-culture with chondrocytes during chondrogenesis. J. Fischer1, A. Dickhurst2, M. Rickert2, W. Richter3.
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Introduction: Bone marrow-derived mesenchymal stem cells (MSC) are a promising cell source for cell-based cartilage repair. A yet unsolved problem is the unwanted upregulation of hypertrophic markers such as alkaline phosphatase (ALP) and collagen type X during in vitro chondrogenesis of MSC. After ectopic transplantation into SCID mice transient calcified cartilage is formed, whereas articular chondrocytes form stable ectopic cartilage without calcification. Aim of this study was to address whether articular chondrocytes have the capacity to suppress undesired hypertrophy in co-culture with differentiating BMSC.

Methods and Materials: Differentiation of MSC (n= 5-7 donors) was induced in chondrogenic medium which had or had not been conditioned by parallel chondrocyte pellets. Alternatively, MSC were mixed with chondrocytes (1:1 and 1:2) and cultured within the same pellet for 6 weeks. Following in vitro differentiation, pellets were transplanted subcutaneously into SCID mice.

Results: The gene expression ratio of COL2A1 versus COL10A1 was significantly enhanced by chondrocyte-conditioned medium (p<0.005; 2.7-fold), while the ALP gene expression and ALP activity was significantly reduced (p<0.05; 3.5-fold and 1.9-fold, respectively). In correlation to lower ALP levels explants differentiated in the presence of conditioned medium showed markedly reduced calcification in vivo. A dose-dependent suppression of ALP activity and in vivo calcification occurred for pellets composed of BMSC and chondrocytes.

Conclusions: In conclusion, secretion of soluble factors by chondrocytes and direct co-culture suppressed hypertrophy and stabilized the chondrogenic phenotype of BMSC in vivo. Current studies now focus on the identification of the active factors that can decrease or prevent BMSC hypertrophy during chondrogenesis.

9.3.1 Rehabilitation after matrix-associated autologous chondrocyte implantation (MACI) on the femoral condyle. What is the optimal time of full weight bearing? B. Wondrasch1, S. Marlovits2.
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Introduction: There is no consensus about the optimal time of return to full weight bearing after MACI on the femoral condyle. Periods of non weight bearing or at least of partial weight bearing seem to be necessary in order to protect the graft in the first time after surgery. Concerning the biochemical and biomechanical properties of healthy cartilage unloading seems to be not physiological. We performed a prospective, randomized, controlled study which compares two rehabilitation protocols with different weight bearing restrictions.

Methods and Materials: In this study 30 patients (11 females, 19 males, aged between 22-56 years) with traumatic lesions of the cartilage of the femoral condyle (mean defect size 5.1 cm²) were treated with MACI and were divided into two groups (A,B). Both groups underwent the same rehabilitation protocol with the main focus on range of motion (ROM), strengthening and neuromuscular control. Group A was allowed to full weight bearing after 10 weeks, whereas group B increased to full weight bearing after 7 weeks. Evaluation was performed after 4, 12, 24 and 52 weeks by objective and subjective evaluation (ICRS) and high resolution MRI.

Results: In both groups no increased pain, effusion or graft loss was observed. The MRI score showed good results in both groups, however patients of group B show less bone marrow edema. Comparing the objective and subjective evaluation scores group B achieved higher levels and better results.

Conclusions: A rehabilitation protocol allowing an earlier return to full weight bearing shows good objective and subjective results. To confirm these findings more studies with higher number of patients should be performed.
9.3.2
A prospective, randomised comparison of traditional and accelerated approaches to post-operative rehabilitation following autologous chondrocyte implantation: two-year clinical outcomes - J. Ebert 1, W.B. Robertson 2, D.G. Lloyd 3, T.R. Ackland 4, M.H. Zheng 5, D.J. Wood 6

Introduction: Policies on post-operative load bearing rehabilitation following autologous chondrocyte implantation (ACI) are varied and the subject remains controversial. We have undertaken a randomised controlled trial (RCT) investigating clinical outcomes in patients who have undergone ACI, following either a ‘traditional’ or ‘accelerated’ return to full weight bearing.

Methods and Materials: Fifty-nine patients with full-thickness medial or lateral femoral condyle defects participated in this RCT. Following ACI, both rehabilitation interventions sought to protect the implant initially, then incrementally increase the load over a 12-week period. Under the ‘accelerated’ protocols patients reached full weight bearing at 8 weeks post-surgery, compared to 11 weeks for the ‘traditional’ group. Clinical outcomes were assessed at 3-, 6-, 12- and 24-months post-surgery.

Results: Although patients in the ‘accelerated’ group reported significantly less pain and symptoms (p<0.05), and displayed superior functional scores (six minute walk distance and daily activity level) at 3-months post-surgery (p<0.05) when compared to the ‘traditional’ group, there were no significant differences at 24-months post-surgery. No patient suffered any adverse effect to the implant as assessed by MRI at 3-months, as a result of the rehabilitation protocols employed.

Conclusions: The ‘accelerated’ load bearing approach that reduced the length of time spent ambulating on crutches produced comparable outcomes at 24-months post-surgery, without compromising graft integrity. This ‘accelerated’ approach is safe and effective, and demonstrated a faster return to normal function post-surgery, with reduced pain and symptoms throughout, and immediately proceeding the rehabilitation program.

9.3.3
Rehabilitation Program After Mesenchymal Stem Cells Implantation For Full Thickness Cartilage Lesions - L. Boldrini 1, L. Bathan 2, A.W. Gobbi 1

Introduction: The surgical one-step procedure of Mesenchymal Stem Cell (MSC) implantation represents a promising alternative to cartilage transplantation in the treatment of full thickness cartilage lesions and permit a significant reduction of operating time and related costs. The rehabilitation program is crucial to optimise the results of the surgery. It promotes the ideal physical environment for MSC to differentiate into articular cartilage-like cells, leading to development of a durable cartilage repair.

Methods and Materials: We followed prospectively five patients with Gr.III or Gr. IV chondral lesions treated with one step procedure of full thickness cartilage lesion repair. The rehabilitation protocol is similar to that used after cartilage transplantation. The protocol follows precise functional criteria and objectives to be achieved in various stages of rehabilitation. The timing and modalities of exercises are determined by the size and location of the lesion. IKDC, KOOS, Lysholm and Tegner scores were collected at pre-op and every 6 months post-operatively.

Results: Five patients with a mean age of 46.6 have included in the study and followed-up for 2 years. The follow-up at 24 months showed an improvements in all scores. Mean values improved from the study and followed-up for 2 years. The follow-up at 24 months showed an improvement in all scores. Mean values improved from the study and followed-up for 2 years.

Conclusions: A specific rehabilitation protocol for one step MSC procedure is important in achieving good clinical results.

9.3.4
Western Australian cartilage repair registry: A multicentre registry of patients undergoing matrix-induced autologous chondrocyte implantation (MACI) in Australia - J.J. Woodhouse 1, D.J. Wood 2, B.K. Joss 3, J.R. Ebert 1, C. Willers 3

Introduction: The Western Australia Cartilage Repair Registry (WACRR) has been established to evaluate the clinical outcome of patients undergoing MACI.

Methods and Materials: Between 2002 and 2008, Australian surgeons maintained a registry of patients treated with MACI. A total of 416 patients who underwent MACI surgery have been identified. To date, 241 patients have completed patient satisfaction questionnaires and surgical data collected. SPSS software (SPSS, Version 11.5, SPSS Inc., USA) was used to perform statistical analysis.

Results: Mean defect size measured 6.5 cm². 33.3% of lesions were isolated to the medial femoral condyle, 8.8% lateral femoral condyle, 18.4% patella, 8.8% tibial groove, 1.2% tibia plateau and 29.5% defects had multiple locations. The graft proved successful in 83.3% of cases and deemed to have failed in 15.4%. Re-operation was required in 13.4% of cases, with 4.6% of these considered major operations with either a reimplantation or knee arthroplasty. 78.9% of patients reported a satisfied outcome and 21.1% were dissatisfied. The ‘accelerated’ load bearing approach that reduced the length of time spent ambulating on crutches produced comparable outcomes at 24-months post-surgery, without compromising graft integrity. This ‘accelerated’ approach is safe and effective, and demonstrated a faster return to normal function post-surgery, with reduced pain and symptoms throughout, and immediately proceeding the rehabilitation program.

Conclusions: Initial follow-up of patients in the WACRR has demonstrated MACI is a successful treatment option for cartilage defects with high patient satisfaction. With continuing follow up, the WACRR will assess the long-term patient outcome and associated factors contributing to graft success or failure.

9.3.5
Structure-modifying effects of chondroitin 4 and 6 sulphate on knee osteoarthritis: final results of the STOPP (Study on Osteoarthritis Progression Prevention) - A. Kahan 1, D. Uebelhart 2, F. de Vathaire 3, A. Kahan 1, D. Uebelhart 2, F. de Vathaire 3, L. Bathan 2, A.W. Gobbi 1

Introduction: Slow-acting drugs for the treatment of osteoarthritis (OA) have been classified as symptom-modifying (SYSDOA) or structure-modifying (SMOAD) if they influence joint structure and disease progression. The usual criterion for SMOAD is prospective evaluation of radiographic changes by analysis of minimum joint-space width (mJSW). Structure-modifying effects are suggested in RCTs with diacerein for hip OA, glucosamine sulphate and chondroitin sulphate (CS) for knee OA. STOPP (Study on Osteoarthritis Progression Prevention) aims to establish over 2 years whether CS improves symptoms and delays joint degradation in knee OA.

Methods and Materials: RCT including 622 patients with knee OA, receiving either Bosom CS (n=309) or placebo (n=313) once daily for 2 years. X-Rays of target knee were taken using Lyon-Schuss view at enrolment, then after 12, 18 and 24 months. MJSW of medial compartment of the tibio-femoral joint was assessed by digital-image analysis. Primary outcome was loss of mJSW over 2 years. CS group (p<0.01). Tolerability and safety were the same in both groups.

Conclusions: STOPP demonstrates that CS Bosom/day acts as a SYSDOA and significantly reduces progression of knee OA, confirming its structure-modifying activity.
9.3.6

Long term clinical assessment following autogenous bone grafting for large volume three dimensional osteochondral defects of the knee.

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Introduction: The purpose is to report the 13-20 year followup of the clinical assessment of patients undergoing autogenous bone grafting of large three dimensional osteochondral defects of the knee joint.

Methods and Materials: There were 39 patients with 41 knee surgeries for bone grafting of lesions of 6-7.5 cubic centimeters. There were 25 patients with osteonecrosis, two of which were iatrogenic. Fourteen patients had osteochondritis dissecans. The follow up was 13 to 20 years with mean of 16 years. Patient followup accomplished by opportunity and intentional research. All patients had pre and post operative extensive clinical electronic data including medical history, physical examination, plain film radiographs, MRI. Video tapes were available on all index surgeries for review and comparison. Second look arthroscopy and biopsy was obtained on 14 patients between 8 weeks and 20 years.

Results: Bone grafts integrated and healed to the physical contour of the graft. MRI showed soft tissue covering graft in all cases at long term followup. Biopsy showed the surface covered with fibrous tissue at eight weeks and subsequently integrated to fibrocartilage with hyaline cartilage at 20 years. Clinical results were satisfactory and joint space was preserved in all but those with existing significant degenerative arthritis.

Conclusions: Autogenous bone grafting is an reasonable alternative treatment for patients with large volume three dimensional defects of the knee joint. The bone grafts healed and integrated and the surface repair was sustained in all showing fibrocartilage and hyaline cartilage at 20 years. The clinical results were satisfactory except en face of significant degenerative arthritis.

9.3.7

Sports participation and patient perspective after cartilage repair of the knee: a comparison between characterized chondrocyte implantation and microfracture; results after three years

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Introduction: To evaluate physical activity and sports participation in a patient cohort involved in the TiGTo18EXT trial comparing Characterized Chondrocyte Implantation (CCI) versus microfracture (MF) in treating knee cartilage defects.

Methods and Materials: Ninety-three Dutch-speaking study participants (CCI n=46 and MF n=47) were followed-up for three years. Through questionnaires, sports activity was measured with modified Baecke Sports Index (MBSI) via blinded telephone survey and Activity Rating Scales (ARS). Clinical outcome of sport participation was evaluated with the KOOS Sports domain. Mann-Whitney U-test was used to compare both treatment groups, and Spearman’s test for correlation between measurements.

Results: Available data from patients at 36 months with the MBSI, KOOS Sports domain and ARS was 83, 53, and 80, respectively. The MBSI (median IQR: 67.5% vs. 72.7% p = 0.73) and ARS scores (median improvement from baseline 0.00 vs. -3.0, p = 0.577) did not differ significantly between groups, thus showing similar activity levels in running and other sport activities. For the KOOS Sports domain, median change from baseline was 35.90 for CCI group vs. 19.20 for MF group (C.I.95%: -16.28,57, p = 0.0047). Correlation between MBSI and ARS was moderate (r = 0.34, p = 0.006) infer that both questionnaires measure a similar construct for physical activity level while correlation between MBSI and KOOS Sports was low (r = 0.179, p = 0.21).

Conclusions: Three years after treatment, the sports participation was comparable in both groups. Although during sports activities patients seem to encounter fewer problems after CCI, as suggested by the KOOS Sports results.

9.3.8

Can arthroscopic or histological scores predict the outcome of Autologous Chondrocyte Implantation?

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Introduction: We set out to investigate whether either the macroscopic or histological appearance of a chondral defect treated with Autologous Chondrocyte Implantation (ACI) can predict clinical outcome.

Methods and Materials: A cohort of patients undergoing ACI in the knee was prospectively investigated. The Lysholm score was used to assess knee function preoperatively and at yearly intervals post-operatively. One year after ACI, arthroscopy was performed and the area treated was assessed macroscopically and histologically. Spearman’s rank correlation was used to compare clinical, macroscopic and histological scores. Statistical significance was set at 0.05.

Results: 29 patients who underwent ACI in the knee between August 1999 and December 2002 had macroscopic and histological assessment at one year. Clinical follow-up was between 2 and 8 years, (median 6 years.) Spearman’s rank correlation coefficient was calculated for arthroscopic and histological scores versus Lysholm score at 1 year, 2 years, and also follow-up, and between arthroscopic and histological scores. The results of this analysis showed that there was no statistically significant correlation between these variables.

Conclusions: This analysis shows that there is no evidence of a direct relationship between arthroscopic score, histological score and clinical outcome. This suggests that there are likely to be factors other than the quality of repair that influence the outcome of ACI.

9.3.9

ICRS I and ICRS II scoring of human osteochondral biopsies: reader variability and sensitivity to staining method

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Introduction: Various scoring systems have been used to evaluate the degree of osteoarthrits and the quality of cartilage repair tissue, with ICRS I (Mainil-Varlet, 2003) specifically adapted for osteochondral biopsies of cartilage repair. ICRS II is a recently modified version of ICRS I. This study evaluates reader agreement of these scoring systems with two distinct histological stains.

Methods and Materials: Human osteochondral biopsies (N=10), containing normal, degraded, and marrow stimulation repair tissue were formalin fixed. Paraffin sections were stained with Safranin-O and H&E. Sections were scored twice by 3 blinded readers using ICRS I (6 categories, ordinal scale) with images associated to each score, and ICRS II (14 categories, continuous VAS scale) with images for the endpoints and at least 1 mid-point. The Intraclass Correlation Coefficient for Agreement (ICC) was calculated to estimate inter-reader agreement.

Results: For ICRS I, categories in good agreement (ICC>0.60) were Surface (0.71), Matrix (0.63) and Cartilage Mineralization (0.65) while the category Cell Viability (0.01) was the least reliable. For ICRS II, very good agreement (ICC>0.80) was obtained for the categories Tissue Morphology (0.88), Matrix Staining (0.88), Tidemark (0.91) and Overall Assessment (0.81), while Cell Morphology (0.24) and Cartilage Matrix Staining (0.38) were less reliable. Overall, ICRS II had better agreement between readers than ICRS I, and agreement was higher when using the Safranin-O versus H&E staining.

Conclusions: ICRS II provides a more detailed assessment of tissue quality with better agreement between readers versus ICRS I. Safranin-O/Fast Green is preferred to H&E when using these scoring systems.
9.4.1 Comparison of characterized chondrocyte implantation versus microfracture in the treatment of symptomatic full thickness defects of the knee results after three years

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Introduction: Purpose: Characterized chondrocyte implantation (CCI) uses an autologous cartilage cell population capable of making stable cartilage in vivo. Despite comparable short-term improvement after intervention, clinical follow-up was to determine long-term clinical benefit of CCI in the repair of full-thickness knee cartilage lesions.

Methods and Materials: Methods: In a randomized controlled clinical trial comparing CCI to microfracture, patients with single ICRS grade III/IV symptomatic defects of the femoral condyles were randomized to receive either treatment (n=57 vs. n=61, respectively). Clinical improvement was measured up to 36 months using the KOOS, Visual Analogue Scale for knee pain (VAS) and Activity Rating Scale (ARS). Treatment failures and safety were monitored throughout.

Results: Results: At baseline, KOOS was comparable between treatment groups (Mean CCI, 56.30 SD13.61; microfracture, 59.53 SD14.95); improvement from baseline in adjusted mean of the overall KOOS at 36 months was 21.25 SE 3.60 for the CCI group and 15.83 SE 3.48 for the microfracture group. In a mixed linear model (with LOCF imputation), significantly greater improvements were shown for CCI vs. microfracture in change from baseline in all KOOS domains (p-value for the Overall KOOS = 0.0007) except for Sports. Between-group improvements from baseline to month 36 in VAS and ARS scores were similar. For CCI vs. microfracture groups, the percentages of treatment responders (improvement of 10 percentage points or more) were 83% (n = 34 of 41) vs. 62% (n = 31 of 50) on the KOOS and 83% vs. 66% on the VAS. Time to treatment failure was not statistically significant between the groups (n CCI/MF = 7/9). There was no change in safety profiles in comparison to the previous recorded data.

Conclusions: Conclusions: The initial superior structural outcome with CCI after 12 months post-surgery was substantiated by superior clinical benefit at 36 months compared to microfracture.

9.4.2 Long-term durability of functional improvement after treatment with Autologous Chondrocyte Implantation (ACI): a multicenter, observational, non-randomized ICRS subset study

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Introduction: Autologous Chondrocyte Implantation (ACI) for full-thickness lesions of the distal femur has demonstrated good short-to mid-term clinical improvement. However, long-term durability (→ 5 years) of ACI has not been evaluated in US patients to date. The purpose of this study was to determine if patients who improve from baseline to early follow-up will sustain improvement at later follow-up.

Methods and Materials: In a multicenter, observational, within-patient control cohort study, study patients met predefined eligibility criteria before data analyses (full-thickness distal femur lesion(s); modified overall Cincinnati knee scores at baseline and 1-5 year follow-up; ACI before Dec. 31, 1996). Per a priori analysis plan, ACI durability was determined by comparing early (1-5 year outcomes to long-term (6-10 year) outcomes.

Results: 72 patients met eligibility criteria. Patients and defect baseline characteristics were: mean age=37 years; 66% male; mean lesion size=4.3 cm2; low mean baseline overall condition score=3.4 points (poor). Within the 5-years prior to the cartilage biopsy harvest, 68% (49/72) of patients had at least one cartilage repair procedure. At 1-5 year follow-up (mean follow-up=4.6 years), 75% (54/72) improved. At 6-10 years follow-up (mean follow-up=9.2 years), 87% (47/54) of patients who improved at the earlier follow-up period sustained a significant mean improvement in overall condition score of 3.8 points from baseline. In addition, at a mean follow-up of 9.2 years, 69% (50/72) of patients significantly improved in overall condition score from baseline. In total, ACI failed in 12 patients; 75% (9/12) of treatment failures occurred at a mean follow-up of 2.5 years.

Conclusions: Treatment with ACI for large, symptomatic, full-thickness lesions of the distal femur in patients with a history of failed prior surgeries can result in early improvement that is sustained at longer follow-up (up to 10 years) in the majority of patients.

9.4.3 Natural history of cartilage defects in the knee: Patients improve their knee function score over time without cartilage treatment, but fail to recover completely

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Introduction: The natural history of focal cartilage injuries is unknown, and despite a high number of cartilage repair surgeries performed, we do not know if surgery improves the long term outcome.

Methods and Materials: The patients in this study are a subgroup of patients in a previous report of 993 knee arthroscopies. Patients younger than 50 years and with a focal ICRS grade 3-4 injury at the time of the baseline study were included (n=98, 13.8% of patients (n=50) in a follow-up study after 6-7 years. Of these, 2 patients were dead, 12 did not meet while 84 completed the follow up study. At follow up a clinical exam including one leg jumps were performed. The patients completed the following questionnaires: ICRS, Lysholm, Tegner, KOOS Cincinnati, IKDC 2000, SF 36.

Results: The average ICRS functional level, the VAS pain score, and knee self-assessment all improved from baseline (p<0.001), while ICRS activity level decreased (p<0.001) from baseline. A linear regression analysis showed that these changes were independent of age, sex, BMI, area and ICRS depth of the cartilage lesion, localization of the lesion, additional injuries or weather the patient had undergone a cartilage repair at baseline or later. At follow up the average Lysholm score was 75 (SD 20), and Cincinnati score was 73 (SD 22). The knee specific scores correlated well with Lysholm score, while SF 36 mental health and SF 36 Role Emotional subscores showed the lowest correlation.

Conclusions: Patients with cartilage defects in their knee improve their functional score over time with or without cartilage surgery, but their knee function is still impaired. Future randomized controlled trials to study cartilage surgery should include a non-surgical group.
9.4.4
The biological fate of autogenous bone grafting for large volume osteochondral defects of the knee: 13-20 years followup.
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Introduction: The purpose herein is to report the long term (13-20 year) followup on the biological fate of an autogeneous bone graft of a large three dimensional osteochondral defects of the human knee joint documented by plain film radiographs, MRI and opportunistic second look arthroscopy and biopsy.

Methods and Materials: There were 39 patients with 41 knee surgeries for autogenous bone grafting of lesions of 6-75 cubic centimeters. There were 25 patients with osteonecrosis, two of which were iatrogenic. Fourteen patients had osteochondritis dissecans. The followup was between 13 and 20 years with mean of 16 years. Patient followup accomplished by opportunity and intentional research. Video tapes were available on all index surgeries for review and comparison. All had pre and post operative plain film radiographs. MRI was performed on 49 knees. Second look arthroscopy and biopsy was obtained on 14 patients between 8 weeks and 20 years.

Results: Bone grafts integrated and healed to the physical contour of the graft. MRI showed soft tissue covering graft in all cases at long term followup (1-10 years). MRI showed graft covered with fibrous tissue at eight weeks and subsequently converted to fibrocartilage with hyaline cartilage at 20 years. Joint space was preserved in all but those with existing degenerative arthritis which became progressive.

Conclusions: Autogenous bone grafting is an reasonable alternative treatment for patients with large volume three dimensional defects of the knee joint. Contrary to presumption, the bone grafts healed and integrated and the surface repair was sustained showing fibrocartilage and hyaline cartilage at 20 years.

9.4.5
The treatment of varus knee with medial osteoarthritis: open wedge high tibial osteotomy with or without associated cartilage repair procedures
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Introduction: Open Wedge high tibial osteotomy (OWHTO) is a well established technique for the treatment of medial gonarthrosis in physiologically young active patients. Cartilage repair process combining OWHTO and microfractures has been described, while only few studies reported the results combining OWHTO and autologous chondrocyte implantation (ACI). Aims of this study is to verify and compare clinical efficacy of three different treatments: isolated OWHTO, OWHTO associated to microfractures and OWHTO associated to ACI in varus knee with medial osteoarthritis (MOA).

Methods and Materials: 70 patients with MOA and varus knee were studied: 28 were treated with isolated OWHTO, 20 with OWHTO plus microfractures and 22 with OWHTO and arthroscopic ACI. The three groups were statistically homogeneous for gender, age, WOMAC and HHS clinical scores, degree of varus and grade of OA at enrollment. Body mass index (BMI) of each patients was collected. Clinical and imaging evaluation were performed at enrollment, 1, 3, 6,12 months postoperatively and then yearly.

Results: At final followup (78 months) improvements in clinical and radiographs were achieved in all were achieved. However isolated OWHTO and OWHTO plus ACI showed a significant higher improvement than OWHTO with microfractures (p<0.05); without significative statistical differences at final follow-up between these two groups.

Conclusions: At final F-up combined OWHTO/ACI and isolated OWHTO procedures showed similar benefits in recovering articular function and reducing symptoms. The combined procedure OWHTO/microfractures showed less improvement in clinical and radiographical results probably due to the significative superior pre and postoperative medium BMI value.

9.4.6
Joint reconstruction of femoral epiphyseal necrosis by two different scaffolds: Comparative results
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Introduction: We present the comparative results obtained by two different scaffolds engineered with autologous chondrocytes in joint reconstruction of femoral epiphyseal necrosis (ARCO 3b, 4). The technique involved removing the chondromalacic area and recovering the necrotic zone, followed by reconstructing the epithysis with bioacellular cylinders (trufit), lyophilized bone chips soaked in packed stromal cells and platelet gel, and finally, covering with engineered scaffold with autologous chondrocytes.

Methods and Materials: Thirteen patients with a total of 14 femoral epiphyses were treated. In the first 7 cases we used Hyaff-11 as the scaffold; subsequently we replaced it with Condro-Gide because of its better mechanical properties. The two groups had matching etiopathogenesis, lesion size, and age, but the follow-up varied. The analyses performed on engineered scaffolds showed that chondrocytes were viable (viability ranged from 89 to 98%) and expressed the typical hyaline cartilage molecules, in particular collagen type II and aggrecan.

Results: At a mean follow-up of 24 months (18-30) in the Hyaff-11 group and 10. 5 months (6-16) in the Condro-Gide group, the joint lining preserved showed thickening with both groups, and pain resolved in 93% of cases. The only case of failure was in the Hyaff-11 group due to a co-existing rheumatic disease. Functional limitation occurred in 4 cases with Hyaff-11 and 3 cases with Condro-Gide, caused by coxofemoral impingement secondary to the difficulty in reconstructing the normal spherical morphology of the femoral epiphysis.

Conclusions: In conclusion, Condro-Gide facilitated articular reconstruction with results analogous to those of the group treated with Hyaff-11.

9.4.7
Histology of chondral lesions before and after the classical debridement in patients appointed for the matrix-ACI
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Introduction: In the classical ACI technique the subchondral endplate should be preserved during the lesion debridement, but recent matrix-based techniques encourage deeper abrasion of the diseased underlying bone. The presented ongoing study is focused on the histological assessment of chondral lesions before and after the classical lesion debridement in patients that undergo alginate-agarose chondrocyte implantation.

Methods and Materials: The press-fit fixation of pre-sized chondrocyte grafts requires deep conical preparation of the subchondral bed (margins 5mm, centre 8mm). Seven patients with chronic chondral lesions (5 OCD, 1 trauma, 1 osteonecrosis) were involved in the study. They gave their consent for the analysis of surplus material removed during the lesion debridement. One central and one peripheral biopsy were taken from the intact lesion by the Jamshidi needle. After the classical debridement with a ring-curette was performed, another central biopsy was removed. The biopsy samples were decalcified, embedded in paraffin, sliced, stained with hematoxylin-eosin, and microscopically analyzed.

Results: The central parts of lesions were covered with a layer of fibrous tissue containing patches of degenerated cartilage. The calcified cartilage thickness was increased. The tide-mark was mostly broken and multiplied, or even absent. Subchondral sinusoids were opened and some patches of degenerated cartilage remained after the debridement.

Conclusions: This was the first study to provide histological analysis of the exact chondral lesions prior to chondrocyte seeding. Manual curettage was unable to remove all the degenerated tissue without opening the subchondral blood pool. However, before we draw any definite conclusions a higher number of patients is needed.
9.4.8

Functional and radiographic outcome of juvenile osteochondritis dissecans of the knee treated with retroarticular drilling

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Introduction: The purpose of this study was to evaluate the functional and radiographic outcome of retroarticular drilling for patients with juvenile osteochondritis dissecans after 6 months of unsuccessful non-operative treatments.

Methods and Materials: Retroarticular drilling was indicated for patients with osteochondritis dissecans whose articular cartilage of the lesion was intact or demonstrated cartilage softening. A total of 20 osteochondritis dissecans lesions in 12 skeletally immature patients were treated with retroarticular drilling. There were 10 boys and 2 girls with an average age of 12.0 years (range: 9 - 15).

The functional outcomes were evaluated using the Lysholm score at an average follow-up of 2.6 years after drilling and healing of the lesions were confirmed using plain radiographs and MRI.

Results: The average Lysholm score significantly improved postoperatively (from 72.4 to 95.8). All lesions except one healed after the retroarticular healing. Healing was achieved at an average of 3.6 months on plain radiographs and 7.6 months on MRI. According to Hughston's criteria, 16 knees were graded as excellent and 2 knees were graded as good. Seven of 9 patients who had been involved in sports activities returned to their previous activities without reduction of their activity levels within 6 months after the operation.

Conclusions: This study clearly demonstrated that retroarticular drilling was an effective treatment option for patients with stable juvenile osteochondritis dissecans, as proved by their functional and radiographic improvement. We advocate retroarticular drilling for patients with initial non-operative treatment has failed.

9.4.9

Distal realignment and patellar autologous chondrocyte implantation. Mid-term results in a selected population.

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Introduction: The aim of this prospective observational study was to assess the 3-year clinical outcome of distal realignment and patellar autologous chondrocyte implantation (MACI®) in selected patients with patellofemoral malalignment and large, isolated, patellar cartilage lesions.

Methods and Materials: Twelve patients (14 knees; 6 females, 6 males; mean age 31 years) with patellofemoral malalignment (lateralized and tilted patella) and Outerbridge grade III-IV isolated patellar cartilage lesions were treated. All had T1-T5 →20 mm on a preoperative CT scan and a cartilage defect →3 cm². Patients with Outerbridge grade III-IV trochlear cartilage lesions, those with rheumatic, infective or neoplastic conditions, or ligament instability, diabetes or obesity and those aged →40 years were excluded. Follow-up was at 36 months. Patients were enrolled after diagnostic arthroscopy. Cartilage was harvested and sent for culture. After a mean period of 30 days patients underwent transfer of the tibial tuberosity according to Fulkerson associated with a MACI® procedure. Clinical assessment was performed with the Kujala, Lysholm, Tegner and Modified Cincinnati scores. The Patient Satisfaction Survey was administered at 36 months.

Results: No major adverse events were seen nor in the postoperative time period. On histological examination, we found a substantial improvement in knee function, either subjective or objective. The mean Kujala score improved from 56 to 88 at 36 months. Mean Lysholm, Tegner and Cincinnati scores improved respectively from 56, 1.3 and 2.6 pre-op. to 90, 4.1 and 7.1 at 36 months.

Conclusions: The significant clinical improvement support the value of associating distal realignment and autologous chondrocyte implantation in treating large, isolated, patellar cartilage lesions associated with patellofemoral malalignment.

15.1.1

Cartilage repair in the patella and the medial femoral condyle – Topographical differentiation using morphological MRI and biochemical zonal T2 mapping

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Introduction: The objective of the present cross-sectional study was to compare cartilage repair tissue in the patella and cartilage repair tissue in the medial femoral condyle in patients after matrix-associated autologous chondrocyte transplantation (MACIT) using morphological scoring and biochemical zonal T2 mapping.

Methods and Materials: Thirty-four patients treated with MACIT underwent 3T-MRI of the knee. Patients were treated on either patella (n=17) or MFC (n=17) cartilage and were matched by age (patella: 36.3±9.9 years; MFC: 35.2±8.2 years) and post-operative interval (patella: 29.3±21.5 months; MFC: 29.3±21.5 months). For morphological evaluation, the MR observation of cartilage repair tissue (MOCART) score was used. For biochemical assessment, T2-mapping was prepared by a multi-echo spin-echo approach with particular attention to the zonal structure of cartilage.

Results: To assess cartilage repair tissue maturation, patient groups were divided concerning their post-operative follow-up into a short-term (0-12 month), a mid-term (13-24 months) and a long-term (60 months) follow-up group.

Conclusions: Quantitative analysis of cartilage repair tissue properties were performed and matching histological sections of the defect were stained for proteoglycans and collagen types I and II. The T2 and T1p values were found throughout the MFC. The zonal increase in T2 values from deep to superficial was highly significant for control cartilage and significant for local cartilage repair tissue. The occurrence of this increase was the oldest of the post-operative interval, was earlier in the repair tissue of the patella.

Introduction: The goal of this initial study demonstrate that differences in T2 values could be found for healthy control cartilage, as well as cartilage repair tissue, between the patella and the MFC. Using morphological and biochemical topographical cartilage parameters, it is possible. Although this study demonstrates the feasibility of describing differences in T2 relaxation times and zonal patterns between cartilage sites with known different biomechanical properties, the in vivo assessment of these properties of articular cartilage still remains challenging.

15.1.2

Quantitative MR Imaging of Cartilage Repair in a Goat Model

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Introduction: Quantitative magnetic resonance imaging (MRI) allows for non-invasive evaluation of cartilage composition. The transverse relaxation time constant, T2, and the longitudinal relaxation time constant (in the rotating frame), T1p, are related to local collagen and proteoglycan content, respectively. The goal of this study was to correlate T2 and T1p of repair tissue with histological evaluation.

Methods and Materials: Osteochondral defects were created in the medial femoral condyle in 15 goats and treated with a proprietary allograft. Ex-vivo T2p and T2 MR imaging was performed and matching histological sections of the defect were stained for proteoglycans and collagen types I and II. The T2 and T1p values, and histology were evaluated from 4 standardized regions of interest relative to the defect. Statistics were performed to detect differences of T1p, T2, and histological parameters across the different regions. Histologic parameters
were correlated with T2 and T1ρ values.

**Results:** The center of the repair had elevated T1ρ and T2 values and low histological scores (p=0.0002), indicative of immature repair tissue. T1ρ correlated with cell morphology (r=−0.4) and Safranin-O (r=−0.5) at the edge of the defect. T2 correlated with cell morphology (r=−0.6) at the center of the defect.

**Conclusions:** This study utilized a cartilage defect repair model to correlate quantitative MR with histologic measures. The correlation of T1ρ and Safranin-O staining corroborate other studies and the lack of correlation between T2 and collagen I staining suggests no relationship between these variables. This study highlights using MR imaging as an outcome measure following cartilage repair.

### 15.1.4

**Clinical Optical Coherence Tomography and MRI T2 Mapping Show Early Cartilage Degeneration**

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**Introduction:** There is tremendous need for early diagnosis and staging of cartilage injury and degeneration using nondestructive imaging. Clinical translation of OCT and MRI T2 mapping of articular cartilage in human subjects was performed to evaluate diagnostic potential.

**Methods and Materials:** Thirty-one human subjects provided informed consent and underwent 3T MRI (NIH Osteoarthritis Initiative sequences, Siemens) followed by OCT and arthroscopic grading of cartilage during arthroscopic surgery, according to IRB approved protocols. OCT grades: 0 – strong birefringence; 1 – intermediate birefringence; 2 – no birefringence; 3 – irregular articular surface. Arthroscopic grades: 0–normal; 1–softened; 2– partial thickness defect, superficial fissures; 3– full thickness fissures; 4–exposed bone. T2 maps were generated using MRI Mapper (© Beth Israel Deaconess and MIT). OCT grades and superficial T2 values from the central medial femoral condyle were compared to arthroscopic grades as the standard.

**Results:** OCT grade increased with increasing arthroscopic grade (p=0.014). T2 map discriminated between cartilage with intact surfaces and cartilage with surface defects (p=0.012), but did not discriminate between arthroscopic grades 0 (soft) and 1 (firm) in cartilage retaining intact surfaces. Tissue demonstrating clear OCT birefringence trended towards midrange T2 while tissue with high T2 trended towards an irregular surface (p=0.17).

**Conclusions:** Clinical OCT was sensitive to cartilage softening prior to surface disruption and provided microscopic cross-sectional tissue visualization not obtainable with conventional arthroscopy. While MRI T2 mapping was insensitive to cartilage softening in this study, it is noninvasive, shows large areas of cartilage, and discriminated between cartilage with intact surfaces and cartilage with surface or deeper defects.

### 15.1.5

**Minimally Invasive Ultrasound Diagnostics of Cartilage Degeneration**

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**Introduction:** Quantitative ultrasound imaging (QUI) provides methods for evaluating integrity of articular cartilage, diagnosing early osteoarthrosis (OA) and monitoring cartilage repair. In this study, we applied a minimally invasive QUI technique and investigated its ability to detect superficial degeneration of bovine knee articular cartilage.

**Methods and Materials:** Intact (n=13), collagenase-digested (n=6) and mechanically degraded (n=7) osteochondral samples (dia.=25 mm) and custom-made phantoms with different surface roughness (n=8) were imaged using a high resolution QUI system at 40 MHz. For each sample and phantom, the ultrasound reflection coefficient (R), integrated reflection coefficient (IRC) and ultrasound roughness index (URI) were determined. Furthermore, to evaluate the clinical applicability of the intra-articular ultrasound (IAUS) diagnostics, one intact bovine knee joint was investigated ex vivo using a simulated arthroscopic approach.

**Results:** Differences in the surface characteristics of the phantoms were detected by changes in the reflection and surface roughness parameters. Both mechanically and enzymatically induced cartilage degradation were sensitively diagnosed by decreased (p<0.05) reflection (R and IRC) at the cartilage surface. Mechanical degradation was also detected by increased (p<0.05) surface roughness (URI). Based on the results, the quantitative ultrasound imaging could be used for detecting collagen disruption and increased roughness of the articular surface. Successful investigation of a bovine knee joint suggested that the IAUS may enable minimally invasive in vivo evaluation of articular surfaces. The in vivo IAUS could have a great clinical value in diagnostics of joint diseases and e.g. monitoring of cartilage repair.
15.1.6

Magnetic resonance imaging of cartilage repair in the knee: benefits of characterized chondrocyte implantation compared to microfracture.

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Introduction: To evaluate clinically relevant MRI findings in a prospective controlled, clinical trial comparing Characterized Chondrocyte Implantation (CCI) with microfracture (MF) in the treatment of cartilage defects of the knee.

Methods and Materials: Patients with a single symptomatic ICRS grade III – IV lesion of the femoral condyle were randomized to either CCI or MF treatment. Structural repair was assessed using MRI at baseline, 12, 24 and 36 months after surgery by two independent radiologists in 112 patients (51 CCI/ 61 MF). A third reader was adjudicated in case of striking discrepancies. MRIs were evaluated with the modified MOCART. Comparative statistics were used to assess treatment-related differences.

Results: After 36 months, cartilage repair scores remained similar for most MOCART Items in both groups. However, clinically relevant results were noted for 'subchondral bone reaction' and 'level of subchondral bone plate'. Preliminary results from the two independent readers for the adjusted means for subchondral bone plate compared to baseline was observed in both groups, especially in the MF group (CCI/MF = 8.4%/9.1% at baseline, 18.1%/43.8% at 36 months).

Conclusions: These findings indicate that, after three years, overall benefits of characterized chondrocyte implantation compared to microfracture.

15.1.7

Cartilage thickness in the dysplastic hip joint measured by MRI and stereorey before and after periacetabular osteotomy

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Introduction: As periacetabular osteotomy (PAO) is performed on dysplastic hips to prevent osteoarthritic progression, changes in the thickness of the cartilage in the hip joint. This method has been applied to investigate if any changes of the thickness of the cartilage take place after PAO.

Methods and Materials: After signed consent, 22 females and 4 males scheduled for PAO were consecutively included. The patients had median age of 39 (19-53) years. Preoperative, the patients were MR imaged on a 1.5 tesla MRI scanner, and this was repeated one year and 2½ years postoperative. To measure the acetabular and femoral cartilages separately, an ankle traction device was used during MRI. We used four reconstructed images through the centre of the femoral head. On each of the images a grid of 10-15 radial test lines was superimposed and where the test lines intercepted the cartilage, the orthogonal distance through the cartilage was manually measured.

Results: Preoperative, the mean thickness of the acetabular cartilage was 1.40 mm, SD 0.16, one year postoperative 1.47 mm, SD 0.13 and 2½ years postoperative 1.35, SD 0.16. The mean thickness for the femoral cartilage preoperative was 1.38 mm, SD 0.18, one year postoperative 1.43 mm, SD 0.13 and 2½ years postoperative 1.38 mm, SD 0.16.

Conclusions: Cartilage thickness 2½ years postoperative compared to preoperative was unchanged in these patients. This indicates that osteoarthrits in these patients has not progressed after PAO.

15.1.8

Correlation between arthro-MRI and hip arthroscopy for the diagnosis of intra-articular lesions

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Introduction: Femoro-acetabular impingement (FAI) cause mechanical pain and has been described as one of the causes of degenerative hip osteoarthritis (OA). Early diagnosis of this condition is important to prevent or delay hip degeneration. The radiological studies provide essential information to plan the surgical technique to use. Arthro-MRI is currently the preoperative gold standard radiological exam for diagnosis of intra-articular lesions (chondral injuries, labral tears, etc). Aim: to determinate a correlation between arthro-MRI and hip arthroscopy for the diagnosis of chondral injuries and labral tears.

Methods and Materials: We performed a prospective study. 27 patients with FAI diagnosis were enrol (29 hips). We compare the arthro-MRI information with the hip arthroscopy findings.

Results: Arthroscopically we observed 26 labral tears and 3 healthy labrums and the arthro-MRI described 25 labral tears and 2 healthy labrums giving an arthro-MRI sensitivity of 96% and specificity of 67% for chondral lesions and 84% for labral lesions. Regarding CTA - implanted labral tears and 16 normal cartilages giving an arthro-MRI sensitivity of 40% and specificity of 50%.

Conclusions: Arthro-MRI is the preoperative gold standard radiological exam for diagnosis of intra-articular hip lesions but have a poor correlation with arthroscopy finding especially for chondral lesions. The results of arthro-MRI must be carefully analyzed and correlated with the clinical exam in each patient.

15.1.9

Usefulness of Magnetic Resonance Imaging for patellofemoral malalignment evaluation

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Introduction: The imaging study usually requested for patellofemoral malalignment (PFM) is threedimensional computed tomography (CT) imaging. Regardless, it is possible to use magnetic resonance imaging (MRI) at 20 degrees of flexion for the same purpose, presenting advantages for the patients, such as avoiding radiation exposure and evaluating associated injuries. The objective of this study is to evaluate the usefulness of MRI in PFM.

Methods and Materials: Twenty seven MRIs prospectively evaluated in 23 patients with PFM clinical diagnose, measuring tibial tuberosity trochlear groove (TTTG) distance, trochlear, Merchant and Laurin angles. PFM was classified according Fulkerson criteria. Associated chondral, meniscal and ligamentous injuries were evaluated as well. Average age 37.1 (10 to 54) years.

Results: All MRIs presented at least one pathological finding regarding PFM. Average TTTG distance 10,6 (4 to 18) mm. Average trochlear angle 139 (125 to 152). Laurin average angle 12,2 (4 to 18). Merchant average angle -5,5 (-27 to 40). Twenty knees presented pathologic TTTG distance. Twelve knees were classified Fulkerson type I, four type II, three type III, two type IV. Ten knees had trochlear dysplasia. Thirty four associated injuries were found in 66,7% of the cases; 22 (64,7%) chondral injuries, 2 Baker cysts, 1 discoid meniscus, 1 localized villonodular pigmented synovitis, 2 patellar tendinitis, 4 internal meniscal injuries, 1 external meniscal injury and 1 partial ACL rupture.

Conclusions: Knee MRI is a useful diagnostic exam in PFM patients. A14%. For age of this exam is that allows the evaluation of associated injuries, particularly chondral. This will help in the preoperative planning.
15.2.1

Subchondral bone removal and regeneration in debrided and microdrilled trochlear defects in adult rabbits after 6.5 months repair

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Introduction: Revascularization and repair of the subchondral bone plate are important processes in cartilage regeneration. We tested the hypothesis that regeneration of mineralized trabecular bone was more frequently detected in control defects 0.5-1.5 mm below the former bone plate, with a similar 45% BV/TV in the 1 mm-deep ROI in treated and control defects. Treated defects however showed a higher bone surface density (Sv, 12 vs 10 mm-1, p<0.05), indicating a more porous trabecular bone repair. Residual drill holes were more frequently detected in control defects 0.5-1.5 mm below the mineralized surface (p<0.05).

Conclusions: Drilling and debridement removed a significant amount of mineralized tissue. Chitosan-GP/blood implants elicited a more porous trabecular bone repair favoring cartilage regeneration.

15.2.2

Therapeutic Strategy of Third Generation Autologous Chondrocyte Implantation for Osteoarthritis

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Introduction: Because of the limited capacity for repair, abnormal wearing of the articular cartilage, osteoarthritis (OA), is a major clinical problem. Autologous chondrocyte implantation (ACI) is one of the promising choices for cartilage repair. The third generation ACI for cartilage injury has been reported, but there is no study of this technique applying for OA. The purpose of this study is to evaluate the efficacy of the third generation ACI for the rat knee osteoarthritis produced by transaction of anterior cruciate ligament (ACL-T OA).

Methods and Materials: We set ACL-T OA rats into three groups of ACI, collagen implantation only, and sham operation (negative control). We examined human cell derived chondrogenesis with mRNA expression by RT-PCR analysis (human specific collagen type2, SOX9) and with immunofluorescence staining for human specific collagen type 2 antibody and HLA-ABC antibody at week 4. We also examined macroscopic assessment and histological evaluation with toluidine blue at week 6.

Results: The expression in RT-PCR analysis and double staining of collagen type 2 and HLA-ABC were seen only in the ACI group. Macroscopic assessment and histological evaluation shows significant recovery was seen in the ACI group at week 8. At week 26, histological score was still better in the ACI group than the other groups.

Conclusions: We showed that the third generation ACI has an application for knee OA in rat. These results indicate the regenerated cartilage was controlled strongly by the implanted human chondrocytes.

15.2.3

The Use of Gellan Gum Hydrogels for Cartilage Tissue Engineering Applications: In vitro characterization and In vivo evaluation

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Introduction: Gellan gum is an extracellular microbial polysaccharide that forms gels in the presence of metallic ions. In this work, gellan gum hydrogels were tested as cell support systems for cartilage tissue engineering.

Methods and Materials: Gellan gum was processed into different structures and characterized in terms of materials properties. In vitro tests were conducted with human articular chondrocytes encapsulated in gellan gum and histology and real-time PCR analyses performed. In vivo evaluation was performed by implanting gellan gum hydrogels discs subcutaneously in mice. In vivo subcutaneous implantation of gellan gum gels with human articular chondrocytes was performed in nude mice for 4 weeks periods. Histology, glycosaminoglycans quantification, and real-time PCR analysis were performed. Finally, full thickness cartilage defects were created in New Zealand white rabbits. Gellan gum-rabbit adipose stem cells systems were injected in the defects. Histology, real-time PCR analyses were performed to evaluate their performance.

Results: Gellan gum hydrogels possess adequate materials properties for the intended application. In vitro results with human articular chondrocytes revealed adequate gene expression (collagen type II, aggrecan). Subcutaneous in vivo implantation showed good integration with a residual fibrotic capsule. Tests in nude mice revealed active synthesis of hyaline cartilage-like extracellular matrix (collagen type II, aggrecan). In vivo tests in rabbits showed homogenous cell distribution and normal morphology, with good lateral integration with the native cartilage. Pineda scoring evidenced a continuous increase in new tissue quality. Alcian blue revealed sulfated proteoglycans deposition and real-time PCR analyses reinforced these observations.

Conclusions: Gellan gum hydrogels systems are promising therapeutic products for cartilage tissue regeneration.

15.2.4

A new vertebrate model for cartilage repair

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Introduction: Axolotl salamanders regenerate entire limbs after injury. During the process of limb regeneration they also regenerate articular cartilage ad integrum. The objective of this study was to investigate the utility of the axolotl as a vertebrate model to study the repair of large articular cartilage defects. We hypothesized that these amphibians possess the intrinsic ability to fully repair large full thickness lesions in the weight-bearing articular cartilage.

Methods and Materials: Single distal femoral condylar defects were created animals four months of age. At 8, 12, 18 and 24 weeks post surgery, hind limbs were collected for analysis. Limbs were fixed and created animals four months of age. At 8, 12, 18 and 24 weeks post surgery, hind limbs were collected for analysis. Limbs were fixed and prepared for paraffin embedding. H&E and Immunohistochemistry was performed on the sections.

Results: A cellular tissue was present in the intra-articular joint space in normal and operated animals. The identity of this “interzone” tissue is unknown. Throughout the healing process cell proliferation appears to have occurred within the defect. During early time points, cells closest to the joint surface were morphologically similar to those within the “interzone” tissue. At later time points, these cells were progressively replaced by chondrocytes. By 24 weeks post surgery, cartilaginous tissue restored the distal femur structure.

Conclusions: The axolotl can repair large, surgically induced full thickness articular cartilage lesions without formation of a blastema. Some of the cells participating in the repair process originate from the interzone tissue. Additional studies of this interzone tissue may provide new information about articular cartilage repair mechanisms in vertebrates that for some reason are no longer fully functional in mammals.
15.2.5

Cartilage repair outcomes differ following microfracture and drilling in a rabbit model

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Introduction: The purpose of this study was to evaluate the effects of the surgical techniques of microfracture versus drilling, and of hole depth, on long-term osteochondral repair in a rabbit model.

Methods and Materials: Trochlear cartilage defects were prepared bilaterally in 16 skeletally mature rabbits. Microfracture holes were made to a depth of 2 mm, and drill holes made to either 2 mm or 6 mm depth under cooled irrigation. Animals were sacrificed 3 months post-operatively and joints analysed by micro-CT followed by histology and immunohistochemistry.

Results: Quantitative histomorphometry showed that drilling provided superior tissue repair, and had a statistically significant increase in the hyaline (proteoglycan) character versus microfracture as indicated by percent of soft repair tissue positive for Safranin-O and collagen type II staining. This better repair after drilling was correlated to previously reported anecdotal where drilling was seen to provide access to viable marrow without apparent heat necrosis, while microfracture induced substantial osteocyte necrosis and bone compaction which impeded osteochondral repair. Furthermore, deeper drilled holes (6 mm) yielded significantly more fill and more collagen type II tissue repair within the cartilage defects than the shallower holes (2 mm). This could be attributed to the fact that 6 mm drill holes had increased access to trabecular marrow stroma and a potentially greater variety of cell types for cartilage repair from the deep marrow.

Conclusions: Drilling of subchondral bone with enhanced access to marrow compartments may be superior to microfracture as a bone marrow stimulation technique for cartilage repair.

15.2.6

A Novel Hyaluronate-Atelocollagen/β-TCP-Hydroxyapatite Biphasic Scaffold for the Repair of Osteochondral Defect

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Introduction: The authors devised a novel biphasic scaffold which combined hyaluronic acid and atelocollagen for the chondral phase, and hydroxyapatite and β-TCP for osseous phase. The purpose of this paper was to introduce the fabrication and physicochemical characteristics of this novel hybrid scaffold, and assess the in vivo and in vitro results of the biphasic chondral repair scaffold.

Methods and Materials: 1) Fabrication of hyaluronate/ateelocollagen scaffold (chondral phase): Hyaluronates in dried powder form and bovine atelocollagen dried powder form was used for fabrication of scaffold. 2) Fabrication of HA / β-TCP scaffold (osseous phase): Urethane foaming method was used to fabricate porous ceramic scaffold. Hydroxyapatite and β-etricalphosphate were mixed in the proportion of 6 to 4 (w/w). 3) In vitro and in vivo chondrogenesis: The defects were managed in one of the following methods: the defects were filled with cell-biphasic scaffold composite (Group I); only biphasic scaffold was implanted (Group II); the removed osteochondral fragments were placed back to the defect (Group III, positive control); the defects were left empty (Group IV, negative control). Seven rabbits were allocated to each group.

Results: 1) Scaffold characterization: SEM findings demonstrated that chondral phase had loose, well-interconnected honeycomb-like structure with large pore (diameter 100-150 µm), the mean porosity being 93±1.4%. Osseous phase also showed well-interconnected porous structures. The diameter was 500-650 µm and the mean porosity was 74±2%. Biphasic scaffold showed that chondral phase interdigitated into osseous phase. The compressive strength of biphasic scaffold was 3.2±0.18 MPa. Three dimensional microtomography also showed fine porous structure of osseous phase. 2) In vivo chondrogenesis: Gross grading score was highest in group III followed by Group I, Group II and lastly Group IV (negative control). Depression of defect was greatest in Group IV while osteochondral autograft in Group III had least depression, sitting proud of adjacent cartilage in two rabbits. There were three rabbit, two in Group I and one in Group II, which were completely denuded of chondral phase, and osseous phase exposed. The junction to adjacent native cartilage was distinct in rabbits of all groups. ICRS Visual Histological Score was highest in Group III, followed by Group I, II. Group I and Group II had almost equal scores.

Conclusions: Results obtained using a rabbit osteo-chondral defect model suggests that biphasic osteochondral composite using chondral phase consisting of hyaluronate and atelocollagen and osseous phase comprising HA and TCP hold promise for the repair of osteochondral defect although denudation of chondral phase is a potential caveat that should be addressed.
**15.2.8**

Glutamate ecrescence from aggregan: Its role in potentiating pain, and the role of glutamate receptor antagonists in reversing sciatic pain in a rat model.

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**Introduction:** In previous publication (Spine 2005), it has been established that human disc material contains almost millimolar concentrations of neurotransmitter glutamate from aggregan. Glutamate from cartilage may potentiate pain by activating local glutamatergic neurons.

**Methods and Materials:** An epidural miniosmotic pump containing concentrations of glutamate or glutamate plus ionotropic receptor antagonist was placed unilaterally at the L5 level. Von frey fiber testing was performed before and 24 hours and 72 hours later. Animals were sacrificed for dorsal root ganglion and dorsal horn quantitative immunohistochemistry and Western Blots for glutamate receptors.

**Results:** Epidural glutamate infusion causes unilateral nociception maximal at 0.2 mM concentration. Dorsal root ganglion and dorsal horn quantitative immunohistochemistry and Western Blots for glutamate receptors.

**Conclusions:** The exquisite pain associated with cartilage fragments in the epidural space may be related to elevated levels of glutamate within the cartilage from the enzymatic breakdown of aggregan. These effects can be reversed with small doses of specific ionotropic glutamate antagonists. Glutamate excitotoxicity may be responsible for the signs of stress in nerves previously attributed to inflammatory processes. Pain of this sort may occur in other cartilaginous joints.

**15.2.9**

Self-assembling peptide amphiphiles (PA) as an adjunct to microfracture for articular cartilage regeneration

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**Introduction:** Peptide amphiphile (PA) molecules can self-assemble into nanoﬁbers of similar dimension and architecture to collagen ﬁbrils and they can be designed to present a high density of bioactive sites. The purpose of this in vivo study was to evaluate the use of PA scaffolds with and without a TGFbeta-1 binding epitope for hyaline cartilage regeneration in a chondral defect.

**Methods and Materials:** Full-thickness chondral defects were created in rabbit trochlea with microfracture. The treatment groups consisted of: 1) TGFbeta-1 alone; 2) non-active PA + TGFbeta-1; 3) TGFbeta-1 binding PA + TGFbeta-1; and 4) TGFbeta-1 binding PA alone. Samples (10 defects per group) were harvested at 12 weeks and were histologically scored by multiple blinded observers.

**Results:** Macroscopic observation revealed that defects treated with the TGFbeta-1 binding PA showed greater tissue ﬁll and integration with the surrounding cartilage compared to defects treated with growth factor alone or with the non-active PA. Histological scores for the TGFbeta-1 binding PA groups were signiﬁcantly higher (40%) compared to the other groups (p = 0.001). Additionally, both groups showed incorporation of the regenerative tissue with the undisturbed cartilage. There was no signiﬁcant difference between the TGFbeta-1 binding PA with or without growth factor, and both groups demonstrated similar cellular morphologies and glycocalyx collagen type II staining compared to the adjacent native cartilage.

**Conclusions:** This study demonstrates that a growth factor binding PA can signiﬁcantly enhance regeneration even without the use of exogenous growth factors. Self-assembling PA scaffolds implanted in conjunction with microfracture technique increase quality and quantity of hyaline cartilage regeneration.

**15.3.1**

Autologous Chondrocyte Implantation. 10-20 years follow up.

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**Introduction:** We assessed 224 patients (144 male, 80 female) with cartilage lesions of the knee, operated with Autologous Chondrocyte Implantation with perioseum, 10 to 20 years ago.

**Methods and Materials:** The average age of the patients at follow up was 46.1 years while it was 33.5 at the time of the implantation. The average size of the lesion was 5.2 cm2. 26% of the patients had multiple lesions while 40% of those had kissing lesions (11% of total). Lysholm, Tegner-Wallgren and Brittbregt-Peterson questionnaire have been assessed. A comparison has also been undergone to a questionnaire evaluation performed 3.1 years after the implantation.

**Results:** Brittbregt-Peterson score was increased from 4.4 to 26.9 and 31.9 for 7, 3 and 1.9. The Tegner-Wallgren score is 8, while it was 7.3 before the ACL, and 8.4 at 3 years follow up. 73.5% of the patients felt better or the same comparing to the previous follow up while 91% would do the operation again. The results show that ACL is an effective durable treatment for cartilage lesions even to 20 years after the implantation.

**Conclusions:** Although the Tegner-Wallgren score reveals a small reduction of the activity level, this can also be ascribed to the expected decrease of the general activity due to increasing age but also due to decreased activity and interest in competitive sports. However, they are still able to do recreational sports.

**15.3.2**

25 years later: The reliability, validity and responsiveness of the patient administered Lysholm score and Tegner activity scale for anterior cruciate ligament injuries of the knee

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**Introduction:** We determined psychometric properties of the Lysholm score and Tegner activity scale as patient-administered outcomes instruments for ACL injuries. We hypothesized both outcomes measures remain valid 25 years after originally validated as physician-administered.

**Methods and Materials:** This study included 1783 patients, 749 females and 1034 males. Average age was 37 years (range, 18-77). Isolated ACL tears were documented in 593 patients; 1190 patients had concurrent injuries including meniscus pathology and/or cartilage damage. Multiple ligamentous injuries were excluded. For responsiveness, scores were measured preoperatively and at least two years postoperatively. For test-retest, scores were measured at least two years postoperatively and again within four weeks. For criterion validity, patients completed the SF-12 and IKDC score and Lysholm and Tegner instrument. For other analyses, preoperative Lysholm score or Tegner scales were used.

**Results:** There was acceptable test-retest reliability for overall Lysholm score (ICC = 0.94 [95% CI = 0.88 to 0.96]) and Tegner (ICC = 0.82 [95% CI = 0.66 to 0.89]). Minimum detectable change for Lysholm was 8.8 points and for Tegner was 1.4. Lysholm scores demonstrated acceptable internal consistency (Cronbach’s alpha = 0.72). Lysholm scores correlated with IKDC (r = 0.78) and physical function domain of SF-12 (r = 0.43). Tegner scale correlated with physical function domain of SF-12 (r = 0.2) and IKDC (r = 0.22). Both instruments had acceptable floor and ceiling effects; all hypotheses were significant. Both had a large overall effect size. There were no differences between isolated and combined ACL injuries.

**Conclusions:** After 25 years of changes in ACL treatment and rehabilitation, Lysholm and Tegner instruments continue to demonstrate acceptable psychometric parameters when patient-administered versus physician-administered.
Conclusions: in 52% of ACI-p (n=100) and 3.4% of ACI-c (n=29).

Results: detect hypertrophy. Demographic and defect characteristics, and this with our collagen ACI (ACI-c) with adequate follow-up (>1y) to compare hypertrophy-related SSP occurred on SSP for hypertrophy in 100 periosteal ACI (ACI-p) and compared methods and materials: this abstract describes the complications associated with periosteum. This abstract describes our results with the use of a collagen membrane in comparison to a retrospective group of our patients treated with periosteum.

Methods and Materials: Since June 2007, we have exclusively performed all ACI using a collagen membrane. We reviewed our data on SSP for hypertrophy in 100 periosteal ACI (ACI-p) and compared this with our collagen ACI (ACI-c) with adequate follow-up (2-12 years) to detect hypertrophy. Demographic and defect characteristics, and differences in hypertrophy-related SSP were analyzed.

Results: Groups were not significantly different for age (36y), defect number (5.9) and size (4.7cm²). Hypertrophy-related SSP occurred in 52% of ACI-p (n=100) and 3.4% of ACI-c (n=29).

Conclusions: The incidence of hypertrophy decreased from 52.0% to 3.4% with use of a collagen membrane. Even though currently off-label in the US, the decreased morbidity due to fewer SSP has validated our decision to switch to a collagen membrane.

15.3.4 10 years of Cartilage Regeneration with Chondroitin Sulfate and Sodium Hyaluronate

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Introduction: Osteochondral lesions of the knee are the most frequent cause of morbidity of this joint and, by far, the most difficult to treat because it is an intense load joint with great instability.

Methods and Materials: Prospective, longitudinal and research 491 patients, 760 knees with chondromalacia and grades I and II osteoarthritis, with painful articular symptoms, previously treated conventionally with NSAIDs or steroid infiltrations, and who were refractory to theses therapies, were treated.

Results: 491 patients, 760 knees 304 women (61.9%), 187 men (38.1%), aged between 12 and 86 years, with a mean of 45.2 years, were treated. 121 knees (25.0%) were diagnosed as chondromalacia, 182 (39.2%) as grade I osteoarthritis, and 457 (60.1%) as grade II. A visual analog clinical scale (WOMAC) 715/760 knees (94.2%) showed significant immediate improvement and satisfactory evolution for up to 2-5-10 years of follow-up, just two patients showed systemic reactions and only one of them reported pain and a slight volume of the knee.

Conclusions: The treatment of osteochondral lesions by intraarticular application of sodium chondroitin sulfate and sodium hyaluronate, with good results at follow-up, has proven to have a significantly favorable clinical response compared with the conventional treatment. This response has been confirmed with pre- and post-treatment arthroscopic imaging, conventional microscopic examination and immunohistochemical testing (POSITIVE S-100 protein) showing that the damaged cartilage is regenerated in an approximate period of 4 to 18 months, recovering its normal structure (De Novo cartilage) and complete function.

15.3.5 Treatment of chondral defects with AMIC technique (Autologous Matrix Induced Chondrogenesis) compared to AMIC enhanced by concentrated bone marrow

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Introduction: It is well established that cartilage lesions up to 2 cm² can be successfully treated by bone marrow stimulating techniques. AMIC® (Autologous Matrix Induced Chondrogenesis) combines the microstructure technique with the use of a collagen matrix for the treatment of lesions ≥ 2 cm². After microfracture, the defect is covered by a collagen I/III matrix, Chondro-Gide®, which stabilizes and protects the blood clot, containing bone marrow elements, stem cells and growth factors.

Methods and Materials: We present the preliminary data of a prospective randomized clinical study, comparing AMIC® to AMIC® combined with concentrated bone marrow for the treatment of larger defects in the knee (2-8 cm²), with a minimum follow up of 6 months. Clinical evaluation of the results are based on Lysholm Knee Score, IKDC score and VAS pain scale. For each patient MRI has been performed preoperatively as well as 6 and 12 month postoperatively. Moreover, a small fraction of bone marrow samples, both from iliac crest and retrieved from MF, has been processed to isolate, characterize and culture the mesenchymal stem cells population.

Results: Significant differences between pre- and post-operative values have been observed for all patients, but without distinction between the groups. MRI showed a good healing process of the cartilage defects. A difference in term of concentration, surface marker expression and differentiation potential have been found between the two samples, with an enrichment of these features in the iliac crest cells.

Conclusions: AMIC technique alone or combined with bone marrow allow to obtain good results in this kind of chondral defects.

15.3.6 Long term results after cartilage repair of the knee with MACI and AMIC procedures comparing clinical and MRI scores

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Introduction: The aim of our study was to correlate clinical and morphologic results of Matrix-induced chondrocyte implantation (MACI) and Autologous matrix-induced chondrogenesis (AMIC). The aim of our study was to correlate clinical and morphologic results of Matrix-induced chondrocyte implantation (MACI) and Autologous matrix-induced chondrogenesis (AMIC).

Methods and Materials: Clinical assessment was achieved by established scores (ICRS, Cincinnati, Lysholm) at the time of MRI. MRI was performed at 1.5 and 3 Tesla (Philips) with ultra high gradients (85 mT/s/m) to allow for a high SNR and a spatial resolution of (~300µm). Pulse sequences with identical parameters were used. We investigated 28 patients (13 female, 15 male, average age at surgery 49 ± 12 years) treated with either AMIC (15 patients, mean 26 ± 9 months post-op) or MACI (13 patients, mean 91 months ± 7 months post-op). MRI evaluation comprised of the degree of defect filling, surface integrity, integration to the border zone, bone marrow edema, osseous or cartilagenous hypertrophy and joint effusion.

Results: Patients treated with MACI showed a defect filling of → 2/3 in average while patients treated with AMIC showed a defect filling ≤ 50 % in average. Osseous hypertrophy frequently surfaced by a thin lamina of repair tissue was observed in → 50 % (AMIC) and ≤ 30 % (MACI) and proved to be one major predictor for failure of cartilage repair. Subchondral bone marrow edema → 1 cm and joint effusion proved to be present in all cases of persisting/recuring deep chondral defects.

Conclusions: Clinical and MRI-scores best correlate in patients after MACI-procedure while MRI scores in patients treated with AMIC lead to an underestimation of outcome compared to clinical scores.
15.3.7
Zone specific clinical outcomes in the treatment of isolated knee cartilage lesions with Matrix-guided Autologous Chondrocyte Transplantation
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Introduction: Cell based techniques, either with or without matrices as cell carriers, have achieved successful results in the repair of isolated full thickness defects of the knee. However, therapeutic outcomes are dependent on the localization of the treated defect within the knee. The complexity of causal pathologies and high shear forces make it difficult to achieve good results in the patellar-trochlear compartment. Matrix-assisted chondrocyte implantation (MACI) is our standard tissue engineering technique for treatment of large, isolated cartilage lesions. The goals of this prospective study were to evaluate the outcomes of MACI therapy after one and two years, and to assess any difference in outcomes between treatment in the condylar and patellar-trochlear zones of the knee.

Methods and Materials: 86 patients with isolated full thickness cartilage defects (minimum 4 cm2) in the knee were included in a prospective study and treated with MACI. Defects were designated as: medial condyle (MC) n=49; lateral condyle (LC) n=26; patellar-trochlear zone (PT) n=11. Results: In May 2008 results were available for 86 patients at least one year follow-up, and for 56 patients at 2 years follow-up. The changes in outcome scores from baseline to 24 months were: A) Meyer’s score: MC 19 to 23; LC 17 to 22; PT 12 to 17 (p=0.0001). B) Lysholm-Gillquist score: MC 2.5 to 3.6; LC 2.2 to 4.5; PT 2.5 to 4.0 (p=0.0001). C) Lysholm-Gillquist score: MC 12 to 17; LC 12 to 18; PT 13 to 17 (p=0.0001). There was no significant difference in the clinical outcome between the three defect locations. No hypertrophy of the regenerated tissue was observed.

Conclusions: Patients with isolated full thickness cartilage defects in the knee showed highly significant improvements when treated with MACI after 6, 12 and 24 months. Sub analysis of clinical outcomes in different defect location showed no significant differences. Sheer forces in the patellar-trochlear region and cartilage necrosis and bone marrow necrosis. Arthroscopic bone marrow stimulation techniques like microfracture (MFX) provide satisfactory outcomes in the majority of the cases. However, in failure cases, more invasive surgeries such as osteochondral grafting or autologous chondrocyte implantation (ACI) are required. Matrix-assisted autologous trochlear chondrocyte implantation (MACI) allows easy placement of the cell carrier and is our preferred second line therapy. The goals of this study was to evaluate the success of our therapy algorithm with MFX and MACI in a prospective cohort study.

Methods and Materials: 36 patients were included in the first cohort and were treated with arthroscopic debridement and MFX of the talus. Rehabilitation included six weeks partial weight bearing after surgery. The clinical outcome was measured on all patients using the American Orthopaedic Foot & Ankle Society (AOFAS) standardized ankle score before surgery and then 6, 12 and 24 months after surgery. The second cohort included 11 patients whose symptoms persisted after the first six months, and who received a second surgery with deep debridement, bone grafting and MACI. Initially, an ankle arthroscopy was performed to harvest a small cartilage biopsy for in-vitro cell expansion and seeding on a collagen I/III matrix. After four weeks an ankle osteotomy was performed to expose the talus. The necrotic cartilage and bone were removed, the bone defect was filled with a cancellous bone graft from iliac crest and a double layer of MACI was glued on top of the lesion. Postoperative care with 6 weeks partial weight bearing was followed.

Results: At May 2008 results were available for all 36 patients in the first cohort, with at least 18 months follow-up. The AOFAS score changed from a mean of 76 at baseline to a mean of 83 after MFX. The 11 patients with an AOFAS score below 70 6 months after surgery received secondary therapy with MACI. Six months later the mean AOFAS score in this cohort had risen to 88. Arthroscopic release of adhesions in the anterior part of the ankle had to be performed in two early cases.

Conclusions: Arthroscopic MFX of the ankle is a minimally invasive technique with a 77% success rate. However, in patients who have failed to achieve symptomatic relief after MFX, secondary treatment with bone grafting and MACI could enhance the outcome. The replacement of necrotic subchondral bone with cancellous bone seems to play a key role in this therapy.

15.3.8
Results with Autologous Bone Grafting and MACI in the Ankle after failed Microfracture
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Introduction: Cartilage defects of the talus are mainly associated with subchondral lesions and are mostly related to ankle trauma. These lesions are often accompanied by persistent subchondral bone marrow necrosis. Arthroscopic bone marrow stimulation techniques like microfracture (MFX) provide satisfactory outcomes in the majority of the cases. However, in failure cases, more invasive surgeries such as arthroscopic debridement or autologous chondrocyte implantation (ACI) are required. Matrix-assisted autologous trochlear chondrocyte implantation (MACI) allows easy placement of the cell carrier and is our preferred second line therapy. The goal of this study was to evaluate the success of our therapy algorithm with MFX and MACI in a prospective cohort study.

Methods and Materials: 36 patients were included in the first cohort and were treated with arthroscopic debridement and MFX of the talus. Rehabilitation included six weeks partial weight bearing after surgery. The clinical outcome was measured on all patients using the American Orthopaedic Foot & Ankle Society (AOFAS) standardized ankle score before surgery and then 6, 12 and 24 months after surgery. The second cohort included 11 patients whose symptoms persisted after the first six months, and who received a second surgery with deep debridement, bone grafting and MACI. Initially, an ankle arthroscopy was performed to harvest a small cartilage biopsy for in-vitro cell expansion and seeding on a collagen I/III matrix. After four weeks an ankle osteotomy was performed to expose the talus. The necrotic cartilage and bone were removed, the bone defect was filled with a cancellous bone graft from iliac crest and a double layer of MACI was glued on top of the lesion. Postoperative care with 6 weeks partial weight bearing was followed.

Results: At May 2008 results were available for all 36 patients in the first cohort, with at least 18 months follow-up. The AOFAS score changed from a mean of 76 at baseline to a mean of 83 after MFX. The 11 patients with an AOFAS score below 70 6 months after surgery received secondary therapy with MACI. Six months later the mean AOFAS score in this cohort had risen to 88. Arthroscopic release of adhesions in the anterior part of the ankle had to be performed in two early cases.

Conclusions: Arthroscopic MFX of the ankle is a minimally invasive technique with a 77% success rate. However, in patients who have failed to achieve symptomatic relief after MFX, secondary treatment with bone grafting and MACI could enhance the outcome. The replacement of necrotic subchondral bone with cancellous bone seems to play a key role in this therapy.
**15.4.1**

**Collagen type II scaffold coating positively influences composition of in vitro regenerated cartilage**

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**Introduction:** Interaction between chondrocytes and scaffold during the matrix-assisted chondrocyte-implantation (MACI) may influence cartilage quality in vivo. Although several types of collagen are used for these constructs, the ideal consistency remains unclear. We aimed at elucidating this issue by culturing human articular chondrocytes on scaffolds with different types of collagen coating.

**Methods and Materials:** Healthy femoral cartilage of 10 human individuals was digested in 0.1% collagenase. Chondrocytes were expanded, followed by culture on Millipore culture inserts, coated with collagen type I (c1), collagen type II (c2), or no coating (NC). Proteoglycan (PG) content and PG release were measured during culture and cartilage quality was analysed after 28 days of culture, using the Bern score.

**Results:** C2 coating resulted in a higher PG content than culture on NC inserts. The high PG release during culture was higher for C1 than for NC inserts (p<0.004). Histological quality of cartilage was similar, although the average Bern score of collagen coated filters was slightly higher. Notably, chondrocytes cultured on NC demonstrated a tendency to form pellet-like structures.

**Conclusions:** An ideal scaffold composition for MACI implantation may aid in regeneration of hyaline-like cartilage. In vitro culture systems mimicking this procedure, may study the role of various compounds, amongst which the collagens. As C2 coating resulted in a higher PG content, a similar effect may occur in MACI grafts. The relatively high PG content on NC, and the high PG release on C1 scaffolds suggest that different aspects of cartilage turnover may be affected depending on the matrix used.

**15.4.2**

**Novel Nano-Composite Biomaterial for osteochondral tissue engineering: Pilot Clinical Study in 30 patients at 2 years follow up**

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**Introduction:** Osteochondral articular defects represent a key concern in orthopedic surgery. The objective of this pilot clinical study was to test safety and performance of a newly developed type-I collagen-hydroxyapatite nanostructural bio-mimetic osteochondral scaffold which reproduces cartilage-subchondral bone morphology.

**Methods and Materials:** A gradient composite O.C. scaffold, based on type-I collagen-HA, was obtained by nucleating collagen fibrils with hydroxyapatite nanoparticles at physiological conditions. 30 cases (9F, 21M, mean age 29.3 years) with knee osteochondral lesions (8 medial femoral condyle, 5 lateral condyle, 12 patella, 8 femoral troclea) were treated with scaffold implantation from January 2007 to July 2007. The lesions size went from 2 cm2 to 6 cm2. All patients achieved minimum 2 years follow up and were clinically evaluated using the International Repair Cartilage Score. The patients were also evaluated with MRI and analyzed with MOCART score.

**Results:** IKDC objective score improved after 2 years showing a normal or nearly normal knee in 80% of patients. Similar results were obtained with the IKDC subjective score and with Tegner score. Cases with previous surgery had the worst results, while associated surgery did not significantly influence the clinical outcome. 1 case failed and was reoperated. MRI evaluation demonstrated good bone and cartilage formation and only in the failed case no integration of the graft was found.

**Conclusions:** This open one-step surgery was used for the treatment of chondral and osteochondral defects. The results of this technique at short follow-up are very encouraging and show satisfactory results even in big osteochondral defects.

**15.4.3**

**Enhanced proliferation and chondrogenic differentiation of human synoviun-derived cells treated by basic fibroblast growth factor on two dimensional culture**

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**Introduction:** Synoviun-derived cells were demonstrated as a useful cell source for cartilage regeneration. In addition, FGFs have important roles in various differentiation processes of stem cells. In our experiment, we treated various concentration of bFGF on 2-dimensional culture of synoviun-derived cells and evaluated its effect on proliferation and chondrogenesis.

**Methods and Materials:** 1) Human synoviun-derived cells were cultured in media supplemented with each concentration of FGF (0, 0.1, 1, 10, and 100ng). 2) Viable cell number was measured by MTS kit.

**Results:** The expressions of FGF-1, 2, 3, and 4 were analyzed by RT-PCR. To induce chondrogenesis, synoviun-derived cell pellets were produced and cultured with chondrogenic medium. 5) GAG amount in pellets was determined by DMB analysis. 6) Pellet specimens were subjected to Safranin-O staining for detection of proteoglycan and IHC for observation of type-I and II collagen.

**Conclusions:** Exposure of synoviun-derived cells to bFGF increased cell proliferation and enhanced the chondrogenesis. We can elect the 10ng/ml of bFGF as the most effective concentration for cell acquisition and chondrogenesis. In next step, we will find the downstream mechanism of bFGF on proliferation and differentiation of synoviun-derived cells.

**15.4.4**

**Cartilage biopsy from the lesion margin as a cell source for the autologous chondrocyte cultivation**

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**Introduction:** The aim was to determine whether the biopsy from the marginal cartilage, to be removed during the lesion debridement, could be used as a source of cells for the autologous chondrocytes cultivation.

**Methods and Materials:** The study comprised of 14 patients (19-43 years) who were appointed to the knee arthroscopy due to a chronic isolated cartilage lesion on the femoral condyles or troclea. In addition to the routine supratrochlear cartilage biopsy a secondary cartilage harvesting from the lesion margin was performed. The lesions originated from OCD 7, trauma 4, and localized degenerative disease 3; 10 were previously untreated, 3 had bone-marrow stimulation, and one had OATS. The cells isolated from both sources underwent parallel cultivation as primary cultures in monolayer, and afterwards they were seeded into the alginate hydrogel for additional 2 weeks. The expressions of typical cartilage genes encoding for collagen I (COL1), collagen II (COL2), aggrecan (AGR), and versican (VER) were assessed in cells from biopsies, primary cultures, and alginite hydrogels by the real-time RT-PCR. The differentiation indexes (COL2/COL1, AGR/VER) were calculated.

**Results:** The control cells revealed higher COL2/COL1 (3-fold in biopsies, 6-fold in primary cultures, and 7-fold in hydrogel; all non-significant) and AGR/VER (71-fold (p<0.05) in biopsies, 3-fold (p=0.05) in primary cultures, and 35-fold (non-significant) in hydrogel).

**Conclusions:** Chondrocytes harvested from the intact articular surfaces express superior phenotype in comparison to cells from the lesion margin. Since the differences were not equalized even in the alginate hydrogel matrix, the cultivation of autologous chondrocytes from the lesion margins cannot be recommended at present.
15.4.5 Comparison of the mesenchymal progenitor cells derived from different porcine tissues for lineage-specific potential

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Introduction: In terms of Good Manufacturing Practice for tissue engineering, obtaining cells without treatment with collagenase that may compromise xenogenetic material is thought to be ideal. Synovial fluid (SF) has been reported to contain mesenchymal progenitor cells (MPCs) that could be isolated without collagenase, however their chondrogenic potential is unclear. The purpose of this study is to compare the mesenchymal differentiation potential among MPCs derived from porcine synovial membrane (SM), SF, bone marrow (BM), and subcutaneous tissue (Skin).

Methods and Materials: The cells were isolated from SF and BM without collagenase digestion. The cells were also isolated from SM and Skin with collagease digestion. The proliferative properties, and the chondrogenic, osteogenic, and adipogenic differentiative properties of these cells were compared.

Results: All sources of cells proliferated up to passage 10 in a comparable manner. All sources of cells also exhibited mesenchymal multi-lineage differentiation potential. Among them, the size of cell-matrix pellets and mean chondrogenic cell clusters revealed that SM-MPCs exhibited the greatest chondrogenic differentiation potential. Alizarin red staining and measurement of calcium deposition revealed that BM-MPCs had the greatest osteogenic differentiation potential. Oil-red-O staining and the quantification of the eluted Oil-red-O revealed that Skin-MPCs had the greatest adipogenic differentiation potential among MPCs.

Conclusions: These results suggested that while all cell sources exhibited multi-lineage potential, there were source-specific differences in effectiveness to generate specific lineages, and SF-MPCs and BM-MPCs might be ideal for cartilage or bone regeneration, respectively, in terms of both safety and efficacy in that xenogenic collagenase was not required for cell isolation.

15.4.6 Internalization of Q-dots to Mesenchymal Stem Cells by Mortalin

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Introduction: Previously, we've reported successful regeneration of cartilage repair using Q-dots labeling and collagenase aggregates derived from MSCs by three-dimensional culturing using an RWG bioreactor. However, the details of the regeneration are unclear. The purpose of this study is to detect the i-QD-labeled chondrogenic cells transplanted into osteochondral defects of rabbits.

Methods and Materials: We used i-QD-labeled human umbilical vein endothelial cells transduced with lentivirus vector containing human mortalin and suspended in alginate beads. Following this period, cells were analysed for tracing using magnetic resonance imaging (MRI). This way, the actual regenerative role of these labelled cells can be further elucidated.

Results: i-QD-labeled cells generated cell-concentration dependent signal voids in an ex vivo cartilage defect model to evaluate MRI traceability on a clinical 3.0-T MRI scanner. Furthermore, labelled cells were seeded in alginate beads. Following this period, cells were analysed for tracing using magnetic resonance imaging (MRI). This way, the actual regenerative role of these labelled cells can be further elucidated.

Conclusions: These results suggested that while all cell sources exhibited multi-lineage potential, there were source-specific differences in effectiveness to generate specific lineages, and SF-MPCs and BM-MPCs might be ideal for cartilage or bone regeneration, respectively, in terms of both safety and efficacy in that xenogenic collagenase was not required for cell isolation.

15.4.7 Chondrogenesis of Adipose-derived Stem Cells for Cartilage Repair

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Introduction: Adipose-derived stem cells (ASCs) demonstrate the potential to differentiate into cartilage. Compared to widely used bone-marrow-derived stem cells, ASCs are easier to obtain and expand in culture, available in larger quantities and have lower donor-site morbidity. The purpose of this study was to determine whether the combination of transforming growth factor beta 1 (TGF-b1) and bone morphogenetic protein 2 (BMP-2) can promote more chondrogenesis of ASCs compared to TGF-b1 or BMP-2 alone.

Methods and Materials: Human ASCs were obtained from healthy donors undergoing elective cosmetic surgery. Aliquots of 2x105 ASCs were centrifuged at 3,000 g for 10 minutes to obtain pellets which were divided into several groups containing different concentrations of growth factors: TGF-b1 or BMP-2 alone, or combinations of TGF-b1 and BMP-2. Pellets were cultured for 14 days. Alcian Blue and immunohistochemical staining for Collagen Type II, as well as Q PCR for Collagen Type II and Aggrecan, were performed.

Results: Alcian Blue and immunohistochemical staining for Collagen Type II, as well as Q PCR for Collagen Type II and Aggrecan, showed the following: BMP-2 alone promoted less chondrogenesis of ASCs compared to the combination of BMP-2 and TGF-b1. BMP-2 combined with TGF-b1 induced a 4.5 times more Collagen Type II and 2.5 times more Aggrecan expression than TGF-b1 alone.

Conclusions: In vitro, combination of TGF-b1 and BMP-2 yielded more chondrogenesis of ASCs compared to TGF-b1 or BMP-2 alone. Future studies involve examining this system in vivo. ASCs combined with specific growth factors can become an important therapeutic approach in cartilage repair.

15.4.8 Clinically relevant cell tracing using Super Paramagnetic Iron Oxides (SPIO) has no effect on chondrocyte behaviour

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Introduction: Cell based techniques for effective cartilage repair are currently under extensive investigation. Today, chondrocytes are the cell type of choice for use in cartilage repair approaches such as autologous chondrocyte implantation. To verify the safety and efficacy of such approaches it is necessary to determine the fate of these implanted cells. Cell labelling using Super Paramagnetic Iron Oxides (SPIOs) provides the possibility for non-invasive in vivo cell tracing using magnetic resonance imaging (MRI). This way, the actual regenerative role of these labelled cells can be further elucidated.

Methods and Materials: Early passage (P2) human articular chondrocytes were SPIO labelled and redifferentiated for 21 days in alginate beads. Following this period, cells were analysed for expression of cartilage related genes, proteoglycan production and collagen-I and -II protein expression. Furthermore, labelled cells were seeded in an ex vivo cartilage defect model to evaluate MRI traceability on a clinical 3.0-T MRI scanner.

Results: SPIO particles, which remained intracellular during the entire experiment, did not significantly affect any of the cartilage related characteristics of redifferentiated chondrocytes. Furthermore, labelled cells generated cell-concentration dependent signal voids on MRI.

Conclusions: SPIO labelling did not affect chondrocyte viability or redifferentiation capacity. Labelled cells could be detected in an ex vivo cartilage defect model in a cell-concentration dependent manner. We consider SPIO labelling a highly promising tool for clinically applicable cell tracing in vivo in cartilage regenerative medicine.
15.4.9
Is Stem Cell a Good Source for Autologous Chondrocyte Implantation? A Comparative Study
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Introduction: Autologous chondrocyte implantation (ACI) is one of techniques to repair articular cartilage defects of knee.

Methods and Materials: To evaluate the efficacy of two cell-types (autologous, bone marrow stem cell (BMSC) comparing with autologous chondrocyte) in ACI, we evaluated 128 patients who were undergone ACI from whom 100 cases were treated by chondrocyte and 28 cases by BMSC. Clinical outcome was measured 3 months and 1 year after operation using ICRS injury questionnaire, IKDC current health assessment (including SF-36 health survey) for functional health evaluation, and IKDC subjective knee evaluation, Lysholm knee scale, and Tegner activity level scale for pain outcome evaluation.

Results: We found a significant increase in functional health (physical functioning, physical health summary, physical role functioning, social functioning, emotional role functioning, mental health (P < 0.001), and mental health summary (P < 0.024) in time in both cell-type groups. However, none of them showed any significant difference between two cell-type. Moreover, IKDC subjective knee assessment, Lysholm, and Tegner score had a significant improvement (P < 0.001) in time. Although Lysholm score had a significant difference between BMSC and Chondrocyte groups, but the difference was not significant for IKDC, and Tegner scores.

Conclusions: The overall clinical outcome evaluation of patients shows that in both groups the improvement in functional health and body pain is the same. Therefore, we can use BMSC as a good substitute for chondrocyte to solve the problem of limitation in the cell number and prevent any damage to healthy tissue without any increase in the cost of the procedure.

19.1.1
The Role of Immunologic Response in Fresh Osteochondral Allografting of the Knee
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Introduction: Osteochondral allografting is a restorative treatment option for articular cartilage lesions in the knee. The technique involves transplantation of fresh, unmatched osteochondral tissue. Although retrieval rates have been reportedly high by shoe manufacturers, this immunologic response, development of anti-HLA cytotoxic antibodies has been observed in allograft recipients. We hypothesize that post-allograft antibody formation is related to graft size and may impact clinical outcome.

Methods and Materials: We retrospectively compared 42 antibody positive post-allograft patients with 42 antibody negative patients. Average follow-up was 59 months (24-165 months). Groups were matched for age, gender, and BMI, but not disease severity. Mean age was 57 years with 62% being male. Graft area was categorized as small (8-15 cm²), medium (16-30 cm²) or large (>30 cm²). Graft survival and Knee Society function scores were used to measure clinical outcome.

Results: Eighty patients had graft area data. 19 of 27 patients (70%) with small graft area were antibody positive, compared to 10 of 16 (6%) with small graft area (P<0.001). 22 patients (26%) were lost to follow-up. Graft survival rates in the antibody positive and negative groups were 64% and 81%, respectively. Mean post-op Knee Society function scores in surviving antibody positive and antibody negative groups were 90.6 and 83.6 points, respectively.

Conclusions: Antibody development after fresh, unmatched osteochondral allograft transplantation in the knee appears related to graft size. Although likely multifactorial, a trend toward decreased graft survival in antibody positive patients was present. This immunologic phenomenon and its effect on clinical outcomes merits further investigation.

19.1.2
Fresh Osteochondral Allograft Transplantation for Osteochondral Lesions of the Talus
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Introduction: Osteochondral lesions of the talus (OLT) are common sequelae of traumatic injuries of the ankle or osteoarthritis events involving the talar bone. Fresh osteochondral allografting is a biologic restoration technique involving transplantation of anatomically appropriate, orthotopic osteochondral grafts. We report on clinical outcomes of osteochondral allografting for symptomatic OLT, utilizing an anterior surgical approach.

Methods and Materials: Between 1998-2006, osteochondral allografting was performed in 13 ankles in 11 patients with OLT. All involved partial, unipolar grafts of the talar dome, implanted through an anterior approach without osteotomy, under temporary distraction. Clinical evaluation was performed utilizing a 100-point Olerud-Molander Ankle Score (OMAS). Subjective outcome measures included patient questionnaires evaluating pain, function and satisfaction.

Results: 7 Males, 4 females had a mean age of 37 years (range 26-57), 4 lesions involved the right, 5 the left ankle; 2 patients had bilateral involvement. All lesions were unipolar, with 8 affecting the medial, and 5 the lateral talar dome. Patients had an average of 1.4 previous surgeries (range 0-5). Mean follow-up was 38 months (range 24-107). Mean OMAS score was 52 points from OMAS of 100. Of 4 patients who had further surgery, 4 patients recorded excellent (OMAS: 91-100), 2 good (OMAS: 61-90), 3 fair (OMAS: 31-60), 2 poor (OMAS: 0-30) outcomes. All patients completed questionnaires; 91% were satisfied, 82% reported improved pain, 64% improved function.

Conclusions: Osteochondral allografting resulted in increased clinical outcome scores with significant improvement in function and pain with high patient satisfaction. Partial talus osteochondral allografting is a reasonable treatment option for appropriately selected patients with unipolar OLT.

19.1.3
Novel osteochondral allograft for the treatment of shoulder instability with glenoid bone loss
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Introduction: We introduce the distal tibia as a novel osteochondral allograft for glenoid bone deficiency and sought to: 1) determine the thickness of glenoid and allograft cartilage via mapping and volume techniques, 2) to determine the optimal bony configuration for tibia matching to the gelenoid.

Methods and Materials: Ten each fresh cadaveric glenoids and distal tibia allografts were topographically analyzed with a Microscribe which mapped and compared the 3D surface anatomy of the glenoid and distal tibia. Glenoid and tibia allograft articular surface were sectioned at 1.5mm intervals for histologic analysis of chondral thickness. The actual amount of bone remaining versus cartilage loss were determined at both 15% and 30% of glenoids bone loss models.

Results: The lateral aspect of the distal tibia provided a near-anatomic representation of the glenoid bone anatomy, but with a radius of curvature of the glenoid cartilage 25.9 +/- 2.1 mm versus 28.1+/- 2.3 mm for allograft cartilage. Cartilage thickness between the anterior 30% glenoid and lateral 30% tibia were nearly identical. Anterior glenoid bone loss of 15% and 30% corresponded to a total cartilage volume loss of 0.8% and 3.9% of glenoid surface area was 1.44 +/- 0.19 cm³/cm² (range, 1.15-1.88 cm³/cm²). Conclusions: The novel allograft tissue (distal tibia) provides excellent articular conformity of a glenoid bone defect; there is significant cartilage loss with glenoid bone deficiency, more so in the anterior quadrant. These findings may favor the utility of fresh osteochondral allograft for reconstruction of glenoid bone anatomy and cartilage loss.
19.1.4 The effect of long term storage of osteochondral allografts
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Introduction: Storage of articular cartilage allografts have been associated with increased chondrocyte apoptotic markers leading to cartilage breakdown. The aim of this study is to characterize the upregulation of apoptotic and matrix related gene expression following long term storage of osteochondral allografts.

Methods and Materials: Osteochondral allografts were harvested from six whole human distal femurs according to tissue back protocols and placed into allograft storage media at 4°C with 0% FBS. After storage for 35 days (maximum time to implantation), they were assessed for the expression of genes related to apoptosis and ECM degradation using two 100 gene microarrays.

Results: At 35 days, the expression of several proapoptotic genes were significantly elevated above baseline (p<0.05) including Caspases 6 and 7, CD30, CD30 ligand, Fas, Fas ligand, and TNF-alpha. Other proapoptotic genes showed consistently strong expression Apollon/Bruc, Bax, CD40, TRAIL receptor and TNF receptor 2. ECM genes including MMPs 1,2,3,9,10,13 or TIMP-1, 2 or 3 did not show upregulation during the storage period.

Conclusions: The loss of chondrocytes via apoptosis during long term storage in cartilage allografts will likely impair their function in vivo. The use of a TNF inhibitor or anti-TNF agent will likely help to modulate the apoptotic response of stored tissue and increase the viability of stored articular cartilage allografts and may improve clinical outcomes post-implantation. Etanercept is a clinically successful anti-TNF agent and shows great promise to modulate the TNF response of stored tissue.

19.1.5 The treatment of severe chondropathies of the knee: Platelet Rich Plasma vs Hyaluronic Acid
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Introduction: The influence of the growth factors on cartilage repair is not yet widely studied and its application in clinics is still experimental. Platelet Rich Plasma (PRP), a blood derived rich in growth factors, is a promising method for treatment of cartilage defects. Aim of this study is to evaluate and compare the efficacy of PRP and Hyaluronic Acid (HA) i.a. injections for treatment of severe chondropathies of the knee.

Methods and Materials: The study involved 120 patients affected by grade III and IV ICRS degenerative cartilage pathology. 60 symptomatic patients were treated with 3 autologous PRP intra-articular injections and evaluated prospectively. For PRP production were analyzed and TNF production were analyzed. For PRP production were analyzed and TNF production were analyzed.

Results: The comparison between the outcomes of the two groups was statistically significant (p<0.0005) in the 3 KOOS subscales, reporting a superiority of PRP group at any times of F-up. The gap between the scores showed to increase with F-up in all the 3 KOOS subscales.

Conclusions: Autologous PRP injections demonstrated more and longer efficacy than HA injections in reducing pain, symptoms and recovering articular function in patients affected by severe chondropathies of the knee.

19.1.6 Initiation of articular cartilage repair by selective COX-2 inhibition in patients with end stage osteoarthritis: ex vivo evaluation of human cartilage tissue after in vivo treatment.
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Introduction: Several recent in vitro studies demonstrated a direct chondroprotective effect of celecoxib, one of the selective COX-2 inhibitors, treating human osteoarthritic (OA) cartilage. The present study aims to validate these findings in an in vivo (clinical) study, treating patients with severe knee osteoarthritis prior to joint replacement surgery with subsequent ex vivo detailed biochemical evaluation of cartilage and synovial tissue.

Methods and Materials: Knee OA patients were treated (orally) 4 weeks prior to scheduled knee replacement surgery with celecoxib 2dd200mg (n=12), indomethacin 2dd50mg (n=8), or received no treatment (n=8). During surgery cartilage and synovium was collected. Chondrocyte activity, matrix integrity, prostaglandin-E2 (PGE2), MMP-activity, TNFα production were analyzed ex vivo. The study was conducted according to the declaration of Helsinki and received ethical approval in all centers.

Results: Age, gender, and histological grade of cartilage damage were not different between the groups. Cartilage PGE2 production was obtained in the treated groups (both p<0.05) in contrast to the COX-2 inhibition. Celecoxib treated patients showed beneficial effects on proteoglycan synthesis (+17%), -release (-77%), and -content (+15%) compared to the non-treated group (all p<0.05). The chondrocyte group demonstrated a tendency towards a lower content and lower synovial release.

Conclusions: Celecoxib treatment decreased ex vivo release of IL-1β and TNFα by synovial tissue (-82% and -73% resp.; both p<0.01), whereas in the indomethacin group only IL-1β release was decreased (-73%; p<0.01). Both treatments showed a tendency to reduce PGE2. Using this approach we demonstrate in vivo generated cartilage repair activity by celecoxib, in contrast to indomethacin, in treatment of end stage osteoarthritis.

19.1.7 Cell communication between subchondral bone and cartilage controls the pathogenesis of Osteoarthritis; monitoring whole tissue turnover in murine femoral heads ex vivo
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Introduction: The pathophysiology of osteoarthritis involves the whole joint, and is characterized both by cartilage degradation and altered subchondral bone turnover. At present there are neither biological models nor tools that allow investigation of the interactions between osteoblasts, osteoclasts and chondrocytes. Thus, we developed and characterised a femoral head model, enabling us to investigate cell interactions and test novel drug candidates for osteoarthritis.

Methods and Materials: Femoral heads from three, six and nine weeks old mice were isolated and cultured for 10 days in DMEM:F12 in absence (control) or presence of IGF-I [100ng/ml] or OSM [10ng/ml] + TNF-α [20ng/ml]. The conditioned medium was assessed by biochemical markers of bone and cartilage. Microscopic changes were assessed by safranin O staining.

Results: Evaluation of three, six and nine weeks old murine femoral heads resulted in henceforth use of nine weeks old femoral heads. Stimulation with OSM + TNF-α resulted in a significant increase in bone resorption (CTX-I), cartilage degradation (CTX-II) and osteoclast number (TRAP). Stimulation with IGF-I, significantly decreased the osteoclast number and showed decreased bone resorption and cartilage degradation. Cartilage formation (PIINP) increased significantly when stimulated with IGF-I, whereas it decreased when stimulated with OSM + TNF-α.

Conclusions: We have established a whole tissue model for osteoarthritis comprising both cartilage and bone, which is highly responsive to both catabolic and anabolic stimulation. This is useful for testing potential disease-modifying osteoarthritis drugs (DMOADs) interfering with more than one aspect of the pathological situation. Furthermore, it presents a unique opportunity for investigating the communication between cartilage- and bone-cells.
19.1.8
Human femoral bone marrow cells obtained during orthopaedic surgery as a potential cell source for articular cartilage repair: influence of donor sex and age
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Introduction: This study tested the hypothesis that the chondrogenic potential of human bone marrow cells (hBMC) isolated from femoral bone marrow obtained during orthopaedic surgery varies based on the sex and/or age of the donor.

Methods and Materials: hBMC were obtained from 12 males (18-80) and 8 females (22-77). Cumulative population doubling in monolayer culture was measured over a 50-day period. Chondrogenesis was evaluated by standard pellet culture assay using chondrogenic medium in the presence or absence of TGFβ1. After 21 days, macroscopic images of pellets were taken, and area calculated. Paraffin sections were stained with toluidine blue and Safranin O to detect proteoglycans.

Results: Population doubling was significantly higher in male than female cells (p<0.008). The increase in pellet size with addition of TGFβ1 to the media was higher in male than female populations. The change in size significantly decreased with increasing donor age in male hBMC (p<0.036), but not in female hBMC. Toluidine blue staining demonstrated that cells with lacunae, indicative of chondrocyte-like morphology, were more numerous in young hBMC. Strong Safranin O staining was only observed in young males.

Conclusions: This study showed that the sex and age of the donor influences chondrogenesis of hBMC obtained during orthopaedic surgery. Male cells proliferated and underwent chondrogenic differentiation better than female cells, and an age-related decline in chondrogenesis with TGFβ1 stimulation was observed. Identifying possible mechanisms for these differences is important for autologous use of femoral hBMC in articular cartilage repair, and will better tailor treatments to both men and women, regardless of age.

19.1.9
Xenotransplantation in cartilage repair - a new practical outlook
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Introduction: Cartilage damage, caused by trauma or other pathologies, results in pain, and altered quality of life. Since cartilage does not heal spontaneously, joint rehabilitation is based on temporary solutions. Autologous transplantation is currently the accepted technology. However, regenerated tissue is of mixed structure: “hyaline-like” and fibrocartilage, in various proportions. The quest to identify the “right cells” to produce the “right tissue” has led to seek the growth factor as origin to hyaline-producing chondrocytes. The neonatal porcine mandibular condyle has demonstrated the most appropriate capacity to spontaneously generate genuine hyaline cartilage, which withstood an extensive preclinical analysis.

Methods and Materials: A novel chondrocytes cell culture originating from neonatal porcine-derived mandibular condyle is proposed. Cells (MCDC) spontaneously differentiate into mature chondrocytes. The neonatal porcine mandibular condyle has demonstrated the most appropriate capacity to spontaneously generate genuine hyaline cartilage, which withstood an extensive preclinical analysis.

Results: Our goat preclinical studies, have demonstrated that the implanted membrane develops into aggrecaan and type II collagen containing surface tissue. Testing for collagen type I is negative. Six months post implantation the implanted cartilage forms tight contact with theaggrecaan, and the subchondral bone. No signs of immune reaction were observed.

Conclusions: Neonatal porcine-derived chondrocytes-MCDC, spontaneously differentiate into hyaline cartilage producing cells developing cartilage membrane-Cartimove™. Xenotransplantation of Cartimove™ is supposed to generating pure hyaline cartilage while maintaining immune neutrality.

19.2.1
Cartilage degeneration in the goat knee caused by treating localized cartilage defects with metal implants
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Introduction: The purpose of the current study was to investigate the feasibility of the application of defect-size femoral implants for the treatment of localized cartilage defects in a 1-year follow-up model.

Methods and Materials: In 13 goats, a medial femoral condyle defect was created in both knees. Defects were randomly treated by immediate placement of an oxidized zirconium (OxZr) (n=9) or cobalt-chromium (CoCr) implant (n=9) or left untreated (n=8). Six un-operated knee joints served as a control. Animals were killed after 52 weeks. Joints were evaluated macroscopically. Cartilage quality was analyzed microscopically and micrographically and cartilage repair of untreated defects was scored microscopically. GAG content, release and synthesis were measured in tissue and medium. Implant osseointegration was measured by automated histomorphometry.

Results: Cartilage repair score of the defects was 13.3±1.0 out of 24. Articular evaluation scores decreased in untreated defects and in defects treated with either implant (p<0.05). Microscopical, morphological and biochemical analysis showed that the presence of untreated defects and the implants had caused considerable degeneration of medial tibial plateau, and to a lesser extent of the lateral tibial plateau. Implantation of OxZr implant was extensive (39.5±9.9% for OxZr and 42.3±11.1% for CoCr) (p=0.05).

Conclusions: Considerable cartilage degeneration was induced in the articulating cartilage one year after creating an osteochondral defect in the medial femoral condyle. Treating this defect with a small metal implant, made of either OxZr or CoCr, could not prevent this degeneration. Further development of defect-size implants is required to make this a therapy of choice for the treatment of local cartilage defects.

19.2.2
Single site osteochondral resurfacing- an in vivo caprine study
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Introduction: This research evaluated a novel biphasic cartilage repair device (CRD). Unilateral stifle arthrotophy (15 goats) and an osteochondral defect (6mm circular, 6mm deep) were made in the medial femoral condyle. Ten were treated with the CRD and divided for 6 and 12 month survival. Five defects acted as empty controls (6 month survival), Weekly lameness and pre-, post-, and end term radiographs were evaluated. At necropsy all stifles were inspected for gross pathology. Blocked defects underwent histological scoring. Statistics utilized ANOVA’s.

Methods and Materials: There was no local or systemic deposition of foreign material from the device. At six months control and treated defect histology scored 8.3 and 14.6, respectively (out of 25). All control defects had subchondral bone cysts and cartilage defects. There was no device migration on post-operative radiography and treated defects had greater intact cartilage surface. There was a trend for smoother hyaline cartilage grossly as well as significantly less degenerative changes histologically in the adjacent cartilage for the treated defects (p=0.02). Lameness and radiographic scores were not significantly different. At twelve months post-operatively, histological scoring of the CRD treated defects demonstrated improved tissue restoration (histological score 17.2).

Results: Defects treated with the CRD underwent repair more rapidly than controls. No inflammation occurred after implantation demonstrating device safety. Evaluation at six months suggests that this device allows for more rapid resurfacing compared to controls.

Conclusions: Evaluation at twelve months support the durability and sustainability of the CRD promoted repair. The device produced a more rapid repair of the articular surface than controls.
Concentrated bone marrow aspirate improves full-thickness cartilage repair.
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Introduction: Cartilage has a limited capacity to spontaneously heal. Our hypothesis was that bone marrow aspirate concentrate (BMAC) would significantly improve the quality and quantity of repair tissue compared to microfracture alone.

Methods and Materials: Horses (n=10) were anesthetized, and 60 ml of bone marrow was aspirated, then concentrated to 7 ml. Arthroscopically, full-thickness cartilage defects were created on the lateral femoral trochlea and treated with microfracture +/- BMAC clotted with thrombin. Repair was assessed arthroscopically at 16 weeks using the ICRS system. Horses were euthanized at 8 months for 3TMRI evaluations including quantitative T1 and T2 mapping. Synovial fluid sample and osteochondral histology sections (H&E, toluidine blue, safranin-O and fast green, COL2 immunohistochemistry) were scored using the ICRS system. A mixed-effect model was fitted to the data and statistical analysis was performed using ps0.05.

Results: No horses developed inflammation or infection. Recheck arthroscopy showed significantly improved repair in the BMAC site compared to microfracture (mean±SEM: BMAC 8.8±1.2, control 16.2±0.49; p=0.0004). Repair tissue remained improved in the BMAC site at necropsy (mean total histology score±SEM: BMAC 7.1±1.2, control 14±1.1 p=0.0006). MRI evaluation indicated significantly increased GAG and collagen content and improved fill in BMAC sites. Histology supported MRI evaluation of improved percent fill, GAG and collagen content in BMAC grafted defects compared to controls.

Conclusions: BMAC provides a method to improve cartilage repair with respect to filling of the defect as well as GAG and type II collagen content. There are no adverse reactions to BMAC grafting. Further studies are required to determine the durability of BMAC grafts in humans.

Articular cartilage and bone repair using in situ polymerizable biodegradable hydrogel implant
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Introduction: Biodegradable materials in osteochondral and chondral defects can promote the repair of the articular cartilage surface through synchronized implant biodegrading. We applied a biodegradable hydrogel, GelrinC™, made from PEGylated fibrinogen and polyethylene glycol (PEG) to 6 mm diameter osteochondral and chondral defects in a goat knee model.

Methods and Materials: One osteochondral or chondral defect was created in the weight bearing zone of the femoral medial condyle of 64 skeletally mature goats. The hydrogel was polymerized in situ by UV photo-cross-linking, while cartilage regeneration, bone regeneration and hydrogel biodegradation were assessed after 2, 4 and 6 months. Evaluation was based on histology, immunohistochemistry and biomechanics.

Results: Osteochondral and Chondral defects treated with GelrinC™ exhibited regeneration of articular cartilage and bone around the eroding implant starting at 4 months follow-up. Empty defects exhibited fibrocartilage and scar tissue formation.

Conclusions: We demonstrate that GelrinC™ hydrogels with controlled proteolytic responsiveness can promote cartilage repair.

Concentrated bone marrow aspirate improves full-thickness cartilage repair
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Introduction: Cartilage has a limited capacity to spontaneously heal. Our hypothesis was that bone marrow aspirate concentrate (BMAC) would significantly improve the quality and quantity of repair tissue compared to microfracture alone.

Methods and Materials: Horses (n=10) were anesthetized, and 60 ml of bone marrow was aspirated, then concentrated to 7 ml. Arthroscopically, full-thickness cartilage defects were created on the lateral femoral trochlea and treated with microfracture +/- BMAC clotted with thrombin. Repair was assessed arthroscopically at 16 weeks using the ICRS system. Horses were euthanized at 8 months for 3TMRI evaluations including quantitative T1 and T2 mapping. Synovial fluid sample and osteochondral histology sections (H&E, toluidine blue, safranin-O and fast green, COL2 immunohistochemistry) were scored using the ICRS system. A mixed-effect model was fitted to the data and statistical analysis was performed using ps0.05.

Results: No horses developed inflammation or infection. Recheck arthroscopy showed significantly improved repair in the BMAC site compared to microfracture (mean±SEM: BMAC 8.8±1.2, control 16.2±0.49; p=0.0004). Repair tissue remained improved in the BMAC site at necropsy (mean total histology score±SEM: BMAC 7.1±1.2, control 14±1.1 p=0.0006). MRI evaluation indicated significantly increased GAG and collagen content and improved fill in BMAC sites. Histology supported MRI evaluation of improved percent fill, GAG and collagen content in BMAC grafted defects compared to controls.

Conclusions: BMAC provides a method to improve cartilage repair with respect to filling of the defect as well as GAG and type II collagen content. There are no adverse reactions to BMAC grafting. Further studies are required to determine the durability of BMAC grafts in humans.

Matrix assisted chondrocyte implantation (MACI®) improves healing of full thickness cartilage defects in an equine model
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Introduction: This study evaluated implant retention, safety, and efficacy of MACI delivered autologous chondrocytes in an equine model.

Methods and Materials: Cartilage biopsies were obtained from six skeletally-mature horses. Chondrocytes were isolated and expanded and MACI® implants formed by seeding onto type I/III collagen (MAxIC®) membranes. Two 15-mm diameter, full-thickness defects were created in the femoral trochlear cartilage of each horse. One defect was treated with a MACI® implant, and the other was left untreated. At 3 months, animals were assessed by arthroscopic scoring. At 6 months, repair areas were scored for gross and histological appearance, analyzed for composition, and tested mechanically.

Results: MACI® improved arthroscopic scores at 3 mths. At 6 months, gross scores of untreated defects were improved for graft perimeter and basal integration, but not total score. Histologically, MACI® defect scores were improved (17.8±4.2) compared with untreated defects (21.5±2.2). Collagen type II had formed throughout the depth of the defect in MACI® treated samples. Proteoglycan and DNA (cellularity) were significantly higher in MACI® treated defects. Mechanical testing revealed no difference between MACI®-treated and untreated defects.

Conclusions: MACI® implants were retained in the cartilage, did not illicit adverse reaction, showed better integration to surrounding tissues, and contained more proteoglycan than nontreated defects. Type II collagen was evident throughout the repair tissue.
Injectable Hyaluronic Acid and Chondroitin Sulphate Therapy for Large Cartilage Defects - A Porcine Model

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Introduction: The clinical use of hyaluronic acid (HA) and oral chondroitin sulphate (CS) in the treatment of osteoarthritis is well-documented. In-vivo studies of the anti-inflammatory and chondroprotective properties of these 2 compounds are also extensive. Our study investigates the effect of direct intra-articular injections of combinations of HA and CS on cartilage healing.

Methods and Materials: A partial-thickness (without penetration of the subchondral bone) cartilage defect was created in the medial femoral condyle of an adult mini-pig. A total of 27 adult mini-pigs were randomly assigned to 3 groups. The study group was injected with 3 injections of 50mg of soluble CS in 2mL of HA (Synvisc) at weekly intervals. 3 injections of either saline or HA were injected into the knees of the controls. The pigs were sacrificed at 6 and 12 weeks for morphological and histological analysis.

Results: The study groups showed improved cartilage healing both histologically and morphologically at 6 and 12 weeks compared to both controls.

Conclusions: Intra-articular injections of HA and CS is a viable option for treating large cartilage defects. This technique is minimally-invasive, and the possibility of it being performed as an outpatient procedure would be further explored in clinical trials.

Evaluation of a biphasic graft for osteochondral repair in an equine model

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Introduction: Osteochondral defects pose a significant challenge for cartilage repair. Our hypothesis was that a biphasic graft (KNC-CRD) would be safe and demonstrate improved osteochondral repair compared with suture repair.

Methods and Materials: Horses (n=12) were anesthetized and injected in the lateral femoral trochlea. A cylindrical osteochondral defect (10x10mm) was created; the KNC-CRD was hydrated with sternal BMA and press-fit into the defect. A full thickness, 10mm diameter chondral defect was significantly improved with KNC-CRD compared to microfracture.

Results: Statistical analysis was performed using Wilcoxon Rank Sum Test (p≤0.05).

Conclusions: Clinical, radiographic and arthroscopic assessment indicated that there were no adverse reactions to the KNC-CRD device. Recheck arthroscopy showed that the overall repair was significantly improved in the biphasic implant site compared to microfracture (mean±sem: KNC-CRD 3.4±0.2, control 5.4±0.8; p=0.0007).

Articular cartilage repair in a caprine model using a regionally specific collagen/glycosaminoglycan osteochondral scaffold

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Introduction: Chondromimetic is a novel biphasic biological scaffold composed of collagen and glycosaminoglycan. The addition of brushite provides the scaffold with a regionally specific component mimicking both phases of the osteochondral unit. The aim of this study was to show the efficacy of Chondromimetic in repairing an osteochondral defect in a caprine model.

Methods and Materials: Defects were made in the lateral trochlear sulcus (LTS) and medial femoral condyle (MFC) of nine goats. Scaffolds (6x6mm) were inserted into each defect (n=6), while three controls had defects left empty (n=3). All defects were sacrificed at 26 weeks postoperatively. Macroscopic evaluations and quantitative stiffness properties were assessed. Histological sections were stained with Saffrin/0/Fast Green and assessed with the modified Sellers score.

Results: Macroscopically, the repair tissue scored higher in the MFC and LTS (p<0.05) compared to controls. In all defects, the mechanical stiffness was found to be within one standard deviation of native cartilage, except that of the LTS controls. Histologically, the predominant tissue in the cartilage layer was deemed to be hyaline-like in three of six MFC defects, and five of six LTS defects. This was compared to one in three and zero of three in the MFC and LTS controls respectively (p=0.05).

Conclusions: A further group of animals will be sacrificed at one year. At six months, the histology and mechanical properties are encouraging and should continue to improve with time. These results show that Chondromimetic may represent an acceptable alternative to narrow stimulation in the treatment of osteochondral defects.

An ACI-like approach for guided tissue regeneration of the avascular meniscal defect: a preclinical goat study

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Introduction: Successful regeneration of meniscal defects in the avascular zone remains a challenge in orthopedics. ChondroGide® has been developed by Geistlich Pharma AG as a biocompatible, bioresorbable collagen I/III membrane for the Autologous Chondrocyte Implantation and Autologous Matrix Induced Chondrogenesis (ACI-AMIC) technique for cartilage defects. The purpose of this study was to investigate the application of cross-linked collagen membrane ChondroGide®-VN in combination with autologous chondrocytes in ACI-like approach for regeneration of avascular tears in a goat model.

Methods and Materials: Longitudinal incisions were created in the avascular zone of medial meniscus of 36 goats and treated with suture alone, suture+ChondroGide®-VN, and suture+ChondroGide®-VN+autologous chondrocytes. Menisci were explanted after 6 months. Analysis comprised histomorphometric and histological assessment. Detailed evaluation criteria were developed. A double-blinded histology evaluation was performed on five serial sections stained with Alcian Blue, Masson TriChrome and H&E.

Results: Clinical observations revealed normal recovery, and individuals indicated no signs of inflammation irrespective of the groups. Based on defect closure, presence of newly formed connecting tissue within the defect, cell formation and deposition of collagens and proteoglycans, suture alone did not allow for stable healing. The application of ChondroGide®-VN led to an increased healing at 3 months, but decreased after 6 months. The combined application of ChondroGide®-VN with autologous chondrocytes demonstrated healing at 3 and 6 months.

Conclusions: The results demonstrated regeneration of avascular meniscal defect via guided tissue regeneration using ChondroGide®-VN, and emphasized the need for cells to guarantee successful long-term healing.
19.3.2

Improvements in function and activity levels after partial meniscectomy are influenced by specific factors

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Introduction: We determined what specific factors influence longevity of improvements in function and activity following arthroscopic partial meniscectomy (PM).

Methods and Materials: 640 knees S/P isolated PM were studied. 193 had partial lateral meniscectomy (PLM), 342 had partial medial meniscectomy (PMM), and 105 had partial medial and lateral meniscectomy. Average age was 52 years (range, 15-70) with 207 females and 433 males. Concurrent ACL reconstructions or microfracture were excluded. Lysholm function and Tegner activity scores were collected for 8 years after index PM.

Results: For all knees, Lysholm scores improved significantly from preoperative (54) to 1 year postoperative (76) (p<0.001). Lysholm was unchanged from years 1-5. At year 6, average Lysholm decreased to 69, and by year 8 decreased further to 63. Non-degenerative knees had greater improvement than degenerative knees and maintained it longer. PMM patients maintained their improvement at 6-7 years while PLM group showed less improvement and decreased at years 6-7. Tegner activity levels improved significantly from preoperative (3.6) to 1 year postoperative (4.7) (p<0.001). Improvement was maintained at years 2-4. There was no significant difference between preoperative and postoperative scores (p=0.05). The same was true at years 6-8. Degenerative knees had less improvement which further declined at years 6-8.

Conclusions: Patients undergoing PM can expect 4-5 years of improved function and activity. Function continues to improve to 5 years but decreases as activity levels decrease. Delayed treatment or degenerative knees experience decreased function and activity sooner. Meniscectomy provides short term improvement in function and activity, but long term improvement seems unlikely.

19.3.3

Short term outcome after implantation of a novel synthetic scaffold for meniscus tissue regeneration

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Introduction: Meniscus regeneration is a desirable therapeutic approach to restore lost meniscus tissue, thereby potentially preventing long-term articular damage. A novel device, designed to act as a Scaffold for meniscal tissue regeneration in patients with irreparable meniscus tears and meniscal tissue loss, has recently been developed and tested in a multicenter single arm non-randomised pilot study.

Methods and Materials: 52 subjects with irreparable medial or lateral meniscal tears or total meniscal loss extending to the vascularized zone, but intact rim, were treated. In this interim analysis tissue ingrowth post-implantation was assessed at 3 months by contrast enhanced MRI (n=41) and at 12 months by gross examination of the implant. Histological examination of biopsy samples (n=9) collected during re look arthroscopy. Clinical outcomes were assessed with VAS, KOOS and IKDC at baseline, 3, 6 and 12 months.

Results: Mean age was 32.9 ±9.0, 77% were male. Meniscus defect location was 47% anterior, 37% medial and 19 lateral. No device related AE’s were observed. 35.41 (85.4%) subjects showed tissue ingrowth on 3 months MRI. Almost 70% (range, 50-100%) tissue gain was observed at 12 months arthroscopy. All 9 biopsies showed complete re-population, illustrating biocompatibility of the scaffold. Zonal organization with particular topography, cell morphology and ECM characteristics suggested ongoing maturation and remodeling towards meniscus-like tissue.

Conclusions: These preliminary results show improvement for all clinical outcome scores and suggest that this novel scaffold supports tissue ingrowth with potential to differentiate and develop meniscus-like tissue characteristics.

19.3.4

Six-year results of collagen meniscus implants (CMI) emphasizing location and meniscus remaining

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Introduction: We determined meniscus loss and location of CMI placement after partial medial meniscectomy (PMM). At 1-year relook we measured total meniscus surface area coverage (MSAC). We correlated percent of meniscus and lesion function with activity for 6 years. We hypothesized MSAC and location influence function and activity.

Methods and Materials: 114 chronic patients (3-11 prior PMM on involved meniscus) underwent PMM. Randomly one group received CMI. There were 68 PMM controls and 46 CMI. At index surgery, amount and location of meniscus removed was noted. PMM placement was always posterior to the lesion.

Results: CMI were posterior (A), middle (B), or anterior (C). Tissue ingrowth post-implantation was assessed at 3 months by contrast enhanced MRI (n=41), and at 12 months by gross examination of the implant. Lysholm scores were lower in CMI patients (p=0.0069). CMI patients with >60% MSAC had significantly higher Tegner index (0.9) versus controls (0.3), p=0.036. Comparing 24 months to final follow-up, controls had no change for Lysholm (p=0.13) or Tegner (p=0.39), but CMI patients improved significantly over time for Lysholm (p=0.02) and Tegner (p=0.04).

Conclusions: Meniscus loss influenced outcomes 6 years S/P CMI. ABC lesions did worse than AB. Successful CMI patients (>60%) were significantly better than PMM. CMI patients continued to improve, but not PMM.

19.3.5

A new quantitative method to evaluate biomechanical performance of a meniscal implant

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Introduction: One of the meniscus’ functions is to distribute forces over the articular surfaces by increasing contact areas. Total/ partial loss of the meniscus increases the risk of joint degeneration. Although previous studies utilized tibial plateau contact-pressure measurements as a biomechanical indicator of allograft menisci performance, there is a paucity of a quantitative method for the evaluation of such outcomes. Here, such a method was developed and employed on sheep and human cadaveric knees.

Methods and Materials: Contact pressures under the intact meniscus were measured in normal human/sheep cadaveric knees under compression (1200N, 0° flexion). Next, total meniscectomy was performed, and the protocol was repeated with meniscal implants.

Results: Both sheep and human cadaveric experiments demonstrated a good correlation between qualitative and quantitative evaluations of the risk of implant performance, and may be used in the future for design purposes and in other orthopedic configurations.
19.3.6

Stimulation of Bone Marrow Stromal Cells Seeded on a Collagen Meniscus Scaffold

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Introduction: In order to generate a functional replacement meniscus implant that is capable of supporting or substituting the meniscus, biologically active implants having tissue specific properties are required. The collagen meniscus implant (CMIA®) is the first scaffold clinically available for in vivo tissue engineering. In the future, cell seeded scaffolds may enhance scaffold stability and tissue maturation. Bone marrow stromal cells (BMSC) are candidates for these strategies. Therefore, the purpose of this study was to investigate the stimulation of BMSC seeded on a collagen meniscus implant by compression and perfusion.

Methods and Materials: 20-80 ml bone marrow aspirate from the iliac crest were collected from 6 donors during routine surgical exposure of the iliac crest. 10^6 human BMSC were distributed on a collagen meniscus implant (CMIA, Regen Biologics Inc., Franklin Lakes, NJ). After 4 hours incubation, the implants were placed into a 12-well, and 3ml of the before mentioned culture media were added. After 24 hours, the one matrix was harvested for analysis. The other samples were transferred into an alternate well (control) or into a bioreactor system where continuous perfusion (10 ml/min) or perfusion and mechanical stimulation (8 hours of 10% cyclic compression at 0.5Hz) were administered daily. After 24 hours, 7 and 14 days, samples were divided in half and analyzed as follows: The MTS assay was used to investigate the proliferation of BMSC seeded on the scaffold. Cell viability was assessed by a live/dead assay. For the assessment of procollagen III peptide (PIIIP), a RIA assay kit was used.

Results: Proliferation demonstrated a significant increase over time in all groups. The number of viable cells after 24 hours was 45±10% and dropped to 29±4% (mechanical stimulation), 32±3% (perfusion), and 41±8% (static control) after one week and remained significantly elevated after mechanical stimulation.

Conclusions: We suggest the following steps for tissue engineering of a meniscus constructs when using the CMI and BMSC: In a first step, a static culture should be used in order to enhance cell proliferation and differentiation.

19.3.7

Medial bone bridge meniscal transplantation. Can it be done anatomically without compromising the ACL footprint?

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Introduction: Restoration of the meniscal meniscus insertion sites during meniscal allograft transplantation is important. The bone bridge technique maintains the relationship between the meniscal horns but may violate the footprint of the ACL. Purpose: Investigate the anatomical relationship between the ACL insertion and the anterior and posterior meniscal horns to determine feasibility of a bone bridge for medial meniscus transplantation.

Methods and Materials: Meniscal horn attachments and ACL tibial footprints were marked and photographed in eight cadaveric knees. The percentage of the ACL intersected by a bone bridge connecting the meniscal horn insertions was analyzed by averaging five images per specimen. Three different bone block widths were utilized (6, 7 and 8mm). Twenty knee MRIs with unjured menisci and ligaments were obtained using axial Fast Spin Echo Proton Density weighted sequences that were sagittally targeted to intersect both medial meniscus horns. Image analysis was performed using MATLAB R2008a software.

Results: The bone bridge intersected with the ACL at each width in all knees. The percentage of the tibial ACL footprint overlapped by the bone bridge ranged from 6% to 30% (mean=22%±11%). Interpreter reliability was high (ICC=0.96). The MRI analysis confirmed these data.

Conclusions: Our results show that medial bone bridge meniscal transplantation interferes with the tibial ACL footprint. The interference ranges from 6-30% of the ACL footprint area. Our axial MRI sequences allowed us to measure ACL footprint compromise. Additional studies are needed to determine what volume of the ACL can safely be compromised during meniscus bone bridge transplantation of the meniscal meniscus.

19.3.8

Non-invasive measurement of 3D meniscal strain

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Introduction: Meniscal tears are a major contributing factor in knee osteoarthritids, and while repair techniques are progressing there is limited evidence that surgical repair restores load sharing by this tissue. The objective of this work was to develop a method for measurement of three-dimensional strain in the meniscus under physiologic loading.

Methods and Materials: Four healthy fresh frozen ovine knee specimens were harvested, two remained intact while two underwent an arthrootomy to create a radial meniscal tears. In all knees tetrahedral clusters of microspheres (0.5mm) were injected into the anterior and posterior aspects of the meniscal using 20-gauge needles. Joints were loaded to 25% and 100% of body weight (BW) in a 4 degree of freedom CT-compatible pneumatically-driven device. MicroCT imaging studies were done with the joints unloaded, after 1, 3 and 5 minutes of 100% BW loading, at the same time intervals with the joint loaded to 25% BW

Results: For intact meniscus specimens, the average maximum principal strains in the anterior element increased by 21% during loading and decreased by 13% during loading at 25% BW. Specimens with meniscal tears had an increase of only 3.7% during loading and decreased by 3.0% after the transition from 100% to 25% BW. The maximum principal strains were 28% larger in the anterior aspect than the posterior in the healthy meniscus, while specimens with radial tears yielded 4.4% larger strain posteriorly.

Conclusions: These studies show that a radial tear markedly disrupts the normal pattern of meniscal deformation and load sharing. This method may be useful in understanding the impact of meniscal pathologies and the value of specific repair techniques.
19.3.9
Biomechanical Evaluation of Meniscal Transplantation with a High Tibial Osteotomy
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Introduction: A high tibial osteotomy can correct alignment and meniscal transplantation can alleviate the sequelae of a meniscectomy. However, the combination of these treatments is controversial. The purpose of this study is to elucidate the biomechanical relationship between a high tibial osteotomy and meniscal transplantation.

Methods and Materials: Four of 8 cadaver knees have been dissected down to the capsule. The femur was mounted in an MTS machine in extension and a Taylor Spatial (TS) Frame was attached to the tibia. An osteotomy at the level of the tibial tuberosity was performed. Anatomic alignment was determined on radiograph and photograph. Tekscan sensors were placed in medial and lateral compartments and the knee was loaded at 800 N until 60% of force was medial and 40% lateral. This position was determined to be 3 degrees of valgus (normal). The knee was taken from 0 to 130 degrees of valgus with the TS software in a medial meniscus intact, meniscectomized and transplanted state. Meniscectomy was performed by removing a bone block and re-implanting in the transplanted state.

Results: There was a significant decrease in pressures in all degrees of valgus when the intact and transplanted state was compared with the meniscectomized condition. (p<0.05)

Conclusions: A high tibial osteotomy can provide relief in a varus knee with medial compartment pain and a meniscal transplant improves the pressure profile of a meniscectomized knee. Meniscal transplantation can have inherent problems with longevity and healing, but this study shows that there is a significant benefit in combining a high tibial osteotomy with meniscal transplantation.

19.4.1
Interleukin-1 silencing using plasmid and integrating transposon based RNA interference as combinatorial therapy in chondrocyte implantation
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Introduction: This study examined whether IL-1 expression could be controlled in reactive joints by using short hairpin plasmid and ultimately integrating transposon plasmid based gene silencing motifs targeting IL-1.

Methods and Materials: Small interfering RNA’s (siRNA) for IL-1β were screened in chondrocyte cultures stimulated by LPS. Most effective siRNA ribo-oligonucleotides were developed into a plasmid coding a short hairpin RNA (shRNA) targeting IL-1β. Subsequently, the pSH.I.L-1β expression cassette was recombined into the Sleeping Beauty (SB) transposon plasmid upstream of a bicistronic IGF-1 expression/selection cassette. Plasmid based efficacy was determined after electroporation to chondrocytes and puromycin selection, using qPCR and ELISA.

Results: Gene expression for IL-1β and MMP-13 were returned to baseline by several IL-1 silencing siRNAs. IL-1 shRNA loops, with or without IGF-1 gene transduction (bicistronic plasmid expressing IGF-1 and IL-1 shRNA), prolonged matrix restorative effects. Chondrocyte monolayers exposed to 0.25% ropivacaine for 24 hrs → 50% IL-1 shRNA expression. Medium from IL-1 shRNA knockdown trials showed IL-1 reduction after shRNA transduction. Effective IL-1 shRNA and IGF-1 coding motifs were developed into Sleeping Beauty plasmid based vectors for transduction of chondrocyte cultures. Both transgenes were expressed and IL-1 shRNA suppressed IL-1 induction in chondrocyte cultures. Moreover, development of this plasmid by recombination into the Sleeping Beauty (SB) transposon, provided potential for chromosomal integration and selection of chondrocytes resistant to IL-1 effects. Supported by NIH R01 AR055373

19.4.2
How to improve the chondrogenic differentiation potential of adipose derived stem cells: A step forward towards regeneration
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Introduction: Adipose tissue represents an easily available source for stem cells, capable of multilineage differentiation. However, the chondrogenic potential of adipose derived stem cells (ASC) is limited under standard differentiation conditions used for bone marrow derived bone cells or dedifferentiated chondrocyte cultures. Since cell based therapies for cartilage regeneration require high cell numbers, this study evaluates both expansion and differentiation conditions.

Methods and Materials: Isolated human ASC were expanded in 2 different media separately (EGM-2 or ASCExp) or subsequently. Next, microarrays based on chondrogenic, and osteogenic differentiation markers were performed. Both media were supplemented with BMP-6, FGF-2 or both growth factors for up to 5 weeks. The potency of chondrogenic and osteogenic differentiation was measured by investigating the expression of IGF-1, IGF-2, BMP-6, IL-1, IL-6, and MMP-13 were returned to baseline

Conclusions: These data show novel IL-1 knockdown using a plasmid shRNA transduction. Effective IL-1 shRNA and IGF-1 coding motifs were identified. shRNA transduction significantly increased after expansion in ASCExp compared to expansion in EGM-2. Subsequent application of EGM-2 followed by ASCExp resulted in rapid expansion and concomitant preservation of the chondrogenic differentiation potential. Regarding the differentiation conditions, BMP-6 demonstrated high capacity to induce chondrogenesis in pellet cultured ASC, while FGF-2 also in combination with BMP-6 strongly inhibited cartilage matrix synthesis. Comparing fibrin and collagen as scaffolds for cartilage tissue engineering, collagen type II mRNA was significantly increased in ASC when cultured in fibrin compared to the collagen scaffold.

Conclusions: In conclusion, the present study demonstrates that expansion and differentiation conditions significantly influence the chondrogenic potential of ASC.

19.4.3
Apoptosis and mitochondrial dysfunction in human chondrocytes following exposure to local anesthetics
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Introduction: Intra-articular local anesthetics are used widely. However, the effect of local anesthetic protein levels has been reported following local anesthetic exposure in human/animal cells. The purpose of this study was to investigate the mechanism of anesthetic toxicity and its effect upon mitochondrial function/cell death in OA chondrocytes.

Methods and Materials: Primary chondrocyte cultures were generated from OA patients undergoing TKA. Cells were treated for 1 hr with 2%, 1%, 0.5% lidocaine; 0.5% and 0.25% bupivacaine; and 0.5% and 0.2% ropivacaine. Control cultures were exposed to saline under the same conditions. After exposure, cells were immediately lysed or placed in normal culture medium for 120 hrs. Total DNA was analyzed for mitochondrial DNA (mtDNA) damage. ATP levels were measured and mitochondrial protein levels were evaluated by Western blot. Cell death was evaluated by flow cytometry, DAPI staining.

Results: After 24 hr exposure, 2% lidocaine demonstrated significant chondrotoxicity with cell loss due to massive necrosis. Exposure to 1% lidocaine and 0.5% bupivacaine resulted in 20-30% viability decrease at 24 hrs, while 0.5% lidocaine, 0.25% bupivacaine, and ropivicaine (0.5% and 0.2%) showed chondrotoxicity viability no different from saline controls. Flow cytometry analysis at 120 h revealed a startling decrease in viability with an increase in apoptosis at all analyzed concentrations of lidocaine, bupivacaine and ropivicaine, except 0.2% ropivicaine. Chondrocyte apoptosis was shown to result from mitochondrial dysfunction. Lidocaine and bupivacaine decreased ATP while ropivicaine minimally affected ATP. A similar effect was observed for mitochondrial protein levels. Under mitochondrial protein levels. Under mitochondrial dysfunction and delayed apoptosis
19.4.4

Bupivacaine, Levobupivacaine and Tramadol are cytotoxic to rats' articular cartilage both invivo and invitro.

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Introduction: Intraarticular use of anaesthetic agents is common for postoperative pain relief after arthroscopic knee surgery. In this study, we have evaluated and compared the effects of Bupivacaine, Levobupivacaine and Tramadol both invivo and invitro experimental rat models on articular cartilage and chondrocytes.

Methods and Materials: Invivo Experiment: 1. Injections: Thirty mature Sprague-Dawley rats weighing 230–300 g were randomized into 3 groups. Bupivacaine (Group 1), Levobupivacaine (Group 2) and Tramadol (Group 3) were injected into the right knee and physiological 0.9% saline into the left. The rats were killed, 5 at 48 h and 5 at 10 days from each group after drug administration.

2. Histopathologic Analysis: The specimens were fixed, decalcified and stained with Hematoxylin and Eosin (H&E) and Toluidin Blue. All samples were evaluated histopathologically according to the recommendation of International Cartilage Repair Society's osteoarthritis and cartilage histopathology grading and staging system.

Invitro Experiment: Articular cartilage cells of the rats were cultured and seeded. Cartilage cell seeded samples (104 cells/mL) were incubated in three different anesthetic agents (0.5%; Bupivacaine, Levobupivacaine, and Tramadol respectively. Cell Titer 96TM Nonradioactivity Cell Proliferation (MTS) assay was used to determine the cell density on the samples.

Results: 1. Invivo: There were pathological changes like cartilage hypertrophy, active chronic inflammation with abscess formation, cellular proliferation, focal vertical fissures and focal discontinuity on cartilage matrix at superficial zone in all three groups on the drug injected sides. Although these histopathologic findings were not found to be statistically significant compared to the control group, OA stage and OA score with the control groups (p>0.05), statistically significant higher OARS grade, OA stage and OA scores were detected when compared the Levobupivacaine injected group after 10 days with the Levobupivacaine injected group after 48 hours (p<0.01; p=0.008). 2. Invitro: MTS results showed that 0.5% Tramadol is cytotoxic to rat chondrocyte in vitro after 30 min of exposure. Also the cell number in both Bupivacaine and Levobupivacaine treated wells showed decrease throughout 15, 30 and 60 min exposures.

Conclusions: Our results suggested that Levobupivacaine and Tramadol may be of some help for improving currently used protocols aimed at biological cartilage repair.

19.4.6

Changes in Secreted Proteins of Human Chondrocytes Cultured as Monolayer or in 3D: Implications in tissue engineering.

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Introduction: The main goal of this study was to investigate secreted protein biomarkers during the in vitro re-differentiation of adult human articular chondrocytes, originally isolated from cartilage explants and expanded in culture dishes.

Methods and Materials: Proteins secreted by de-differentiated (monolayers) as well as re-differentiated (spheroids) chondrocytes were analyzed by SDS-PAGE electrophoresis in tandem with MS-protein spectrometry. Identified secreted proteins were compared with proteins in synovial fluids from knees. RT-PCR was used to validate gene expression of corresponding genes in cells cultured under different conditions.

Results: Our results indicate that chondrocytes cultured in 2D and 3D produce and secrete proteins naturally occurring in plasma and synovial fluid such as albumin, transferrin and apolipoproteins. Several matrix components such as laminin, lumican, biglycan, heparan sulphate proteoglycan and hyaluronan were only found in spheroids supernatants. Using antibody arrays we have shown that chondrocytes established in monolayer secrete significantly more leucocyte-activating agents such as MCP-1 and GRO, were as the spheroids. Re-differentiation favors the production of matrix regulating factors like MCSF and other morphogens such as VEGF.

Conclusions: In summary, we have discovered a panel of secreted proteins that are differentially expressed and secreted by chondrocytes during de- and re-differentiation in different growth conditions in serum-free media. We have discovered that albumin is secreted abundantly by chondrocytes during monolayer growth and to a lesser extent during 3D cultures. Our results may help in better understanding of the underlying mechanisms in chondrocytes re-differentiation, and may be of some help for improving currently used protocols aimed at biological cartilage repair.

19.4.5

Periosteal flaps are unneccessary in ACI ? Final in-vitro results

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Introduction: Clinically the second generation of ACI without periosteal flaps for closure of the biochamber is already clinical routine.

The question behind: Is there any biochemical need for the flap or was it’s function only mechanical?

We created a model to analyse the influence of periosteal flap on isolated chondrocytes in a perfusion co-culture system under continuous delivery of the medium.

Methods and Materials: We used fresh bovine cartilage explants and periosteal tissue harvested from commercially slughtered cows. Chondrocytes were isolated and cultured under standard culture conditions in a perfusion system (Minucell & Minutissue, Bad Abbach,Germany) enabling under continuous perfusion either a mono-culture of chondrocytes or a co-culture of chondrocytes with periosteal explants. Both were compared to a chondrocyte monoculture under static conditions without continuous delivery of medium.

For analyses of the collagen types we used immunohistochemical typing, for detection of the cell proliferation DNA-quantification was performed. For measurement of the proteoglycan-content incorporation of radio-labelled sulfate was measured. Production of new collagen was measured with radioactive labelled prolvin and for measurement of the GAG-production the DMB-Assay was used.

Results: Under the influence of the periosteal tissue in the co-culture system chondrocytes proliferation was reduced with dedifferentiation and reduced deposition of collagen-type-II. In addition, we found a reduced incorporation of radio-labelled prolvin and sulfate and reduced GAG-synthesis.

Conclusions: In summary, our results demonstrated that there is no need for a periosteal flap to promote cartilage cell differentiation and proliferation under in-vitro-conditions. This in-vitro analysis supports the clinical use of scaffolds instead of periosteal flaps.

19.4.8

Novel chondroitin sulphation in foetal human knee development

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Introduction: In a previous study (Hayes et al., 2008; J. Histochem. Cytochem. 56: 125) we demonstrated that novel chondroitin sulphate (CS) sulphation motifs on cell-associated proteoglycans (PGs) are putative biomarkers of progenitor/stem cell sub-populations residing within the superficial zone of articular cartilage and that monoclonal antibodies (mAbs) 3B3-1, 7D4 and 4C3 could potentially be used to isolate and purify these cell niche populations. In this study we examined the distribution of novel epitopes in the developing human knee joint.

Methods and Materials: 12-14 week human foetal knee joint rudiments were fixed, processed into paraffin, sectioned and
immunoperoxidase-stained with mAbs 3B3(-), 7D4 and 4C3, counterstained with Haematoxylin and then photographed.

Results: All three CS sulphation motif epitopes localised prominently at sites of incipient articular cartilage formation at a stage before there was any histological evidence of secondary ossification at the epiphysis. Their staining was detectable in very defined regions within the perichondrium; growth plate; fibrocartilage of both meniscus and enthesis; vasculature; and at sites of capillary invasion, with subtle differences in their distribution.

Conclusions: This study showed that these mAbs recognise unique sulphation motifs within the stem/progenitor cell niches of articular cartilage and also a wide range of other musculoskeletal tissues. We hypothesize that the unique sulphation sequences on CS-PGs are involved in regulating cell proliferation and differentiation events through interactions with soluble signalling molecules. We aims to establish which PG core proteins carry the different CS sulphation motifs and identify potential interactions with soluble signalling molecules involved in musculoskeletal tissue development and growth.

19.4.9 Quantitative histomorphometry of collagen types I & II and safranin-O in human osteochondral biopsies
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Introduction: The purpose of this study was to develop reliable collagen and glycosaminoglycan staining methods in sections from human osteochondral biopsies of cartilage repair, and to validate the reproducibility of quantitative histomorphometric measurements by independent readers.

Methods and Materials: Ten 2 mm diameter human osteochondral biopsies were obtained from human cadavers, OA hip arthroplasty (N=6), and human patients 12 months following marrow stimulation-based cartilage repair procedures (N=4). Decalcified osteochondral paraffin sections were stained for Safranin O, or immunostained for collagen type II or collagen type I. Three independent readers performed two distinct blinded quantitative histomorphometric assessments of collagen and Safr-O staining. Inter-reader and intra-reader reliability was estimated using the Intraclass Correlation Coefficient (ICC, where 1.0 is perfect agreement).

Results: Very high intra- and inter-reader agreement was obtained for non-calcified tissue thickness and volume (r>0.96), and percent tissue area stained with Safranin O, or immunostained for collagen type II or collagen type I. Three independent readers performed two distinct blinded quantitative histomorphometric assessments of collagen and Safr-O staining. Inter-reader and intra-reader reliability was estimated using the Intraclass Correlation Coefficient (ICC, where 1.0 is perfect agreement).

Results: Very high intra- and inter-reader agreement was obtained for non-calcified tissue thickness and volume (r>0.96), and percent tissue area stained with Safranin O (r>0.82), collagen type II (r>0.79), and collagen type I (r>0.62) in biopsies where non-calcified tissue thickness varied from 0.94 to 6.31 mm. The group of 10 biopsies showed 58-99% Safranin O+ stain, 42-99% collagen type II-positive tissue, and 0-57% collagen type I-positive tissue. Correlation analyses revealed that samples with >28% collagen type I were depleted of glycosaminoglycan (<72% Safranin O+).

Conclusions: Quantitative histomorphometry of histological and immunohistochemical staining resulted in very high intra- and inter-reader agreement and consistency for tissue thickness, volume, and stained area for matrix molecules directly responsible for cartilage structure and function. These validated methods can be used to objectively assess quality of tissue repair in osteochondral biopsies.