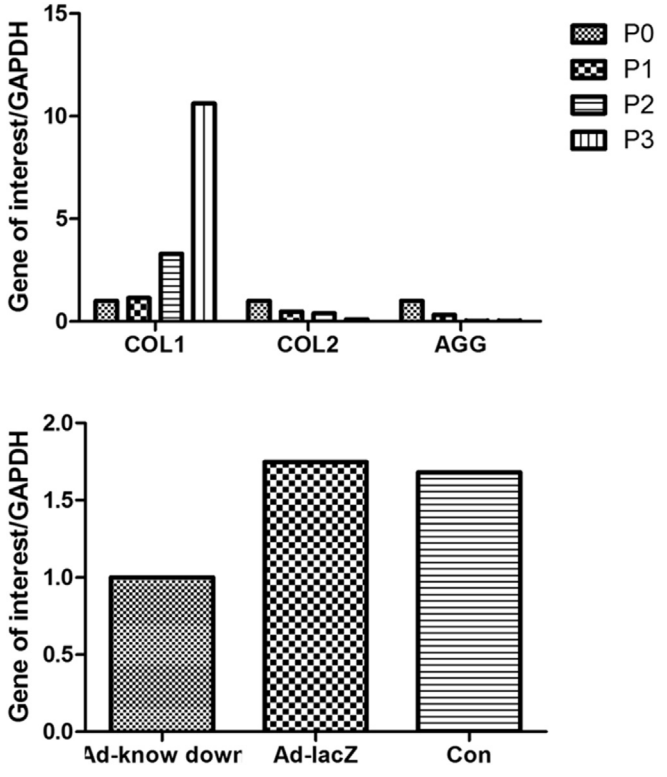
Purpose: To produce a new tool for investigating primary culture and adenoviral infection of chondrocytes from mature mouse articular cartilage (C57BL/6J)
Methods: We used four-month old C57 BL/6J mice as provider and dissected articular cartilage from tibial plateaus and femoral condyles for primary culture, then examined several morphological and biochemical features of different passages to determine which passage is suit for deep investigation. Furthermore, we used adenovirus as powerful gene transfer vector system to achieve gene SREBP-2 knock-down.
Results: The cells from P0, 1 had the typical chondrocytes morphology, with a rounded or polygonal shape, but till P2,3 the cells became dedifferentiation with the shape changed. Alcian-Blue and Immunofluorescence staining showed that chondrocytes from P1 express proteoglycans and collagen II respectively. The results from RealTime-PCR showed collagen II and aggrecan expression decreased with passages. In contrast, collagen I expression was low in primary cultures and became much higher after passages, indicating that this kind of chondrocytes became fibroblasts and not suit for deep investigation. Gene delivery via adenoviral infection was highly efficient and reproducible at 50 MOI for 48 h, and the RT-PCR of gene SREBP-2 proved the effects.
Conclusions: We have developed practical and reliable methods to isolate and culture chondrocytes from articular of mature mouse and to implement acute gene knock-down in these chondrocytes via adenoviral infection. The culture and adenoviral infection of mature mice articular chondrocyte described here should prove a powerful tool for deeper research for virus theory and drug delivery at the cellular or subcellular level.



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UNILATERAL ANTERIOR CROSSBITE INDUCES ABERRANT MINERAL DEPOSITION IN DEGENERATIVE TEMPOROMANDIBULAR CARTILAGE IN RATS

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Purpose: Rrecently, we have developed a unilateral anterior crossbite (UAC) procedure that could induce osteoarthritic (OA)-like changes in the temporomandibular joints (TMJs) cartilage of rats and mice. Herein, we employed imaging, histological, and molecular biology techniques, and also in vitra assay, to investigate whether there is mineral deposition in the UAC induced OA cartilage, what the composition the mineral deposition has, and what a role of the minerals may take in OA cartilage.
Methods: Spraguee-Dawley rats were subjected to a unilateral anterior crossbite (UAC) procedure. Histology, electron microscopy, and energy dispersive spectroscopy (EDS) were used to examine cartilage matrix structures and compositions of mineral deposit in TMJs. Protein and/or RNA expression of modulators of phenotypic markers, mineralization and matrix degradation was analyzed by immunohistochemistry and/or real-time PCR. Synthetic BCP and CPPD crystals were used to stimulate chondrogenic ATDC5 cells for their impact on activities of cellular differentiation and secretion.
Results: The proteoglycan content in UAC cartilage began to decrease as early as 2 weeks post-operation and nearly absent at 20 weeks post-UAC without significant inflammatory response. The collagen arrangement was obviously interrupted and the cartialge thickness reduced from 8 weeks but the thickness of the calcified zone was increased. The expression of Col-II, Col-X, and aggrecan was decreased. The control group chondrocytesunder transmission electron microscopy (TEM) were found in well-delimited lacunae that were surrounded by the tightly-organized collagen fibers. In UAC cartilage, however, collagen fibers were disorganized and fragmented with increasing inter-fibers spaces and were worsened with time. Mineral deposition was observed around the hypertrophic chondrocytes, beginning 2 weeks after the UAC procedure. Those peri-cellular minerals were contained in abundant matrix vesicles (with diameters ranging from 50-200 nm) that were located in close proximity to the cell member of hypertrophic chondrocytes, supporting the cellular origin of the vesicles.
Immunohistochemistry and/or real-time PCR analyses demonstrated biphasic changes in the expression of MGP mRNA in TMJ joints of UAC rat, which increased at 2 and 4 weeks post-operation and decreased after 8 weeks post-operation when compared to age-matched controls. The expression of CD73 and Ank was significantly reduced in TMJ cartilage of UAC group at all time points. The expression of Npp1 was significantly reduced in UAC cartilage at 2 and 4 weeks post-operation. In contrast to the expression of anti-mineralization molecules, the expression of Tnap, which hydrolyzes PPi into Pi to promote the formation of BCP crystals, was up-regulated in the UAC groups at 4, 8, and 12 weeks post-operation, and this effect waned at 20 weeks post-operation. The expression of matrix metalloproteinase, Mmp13, which cleaves type II collagen, was also up-regulated in the UAC groups in the similar time-dependent manner as seen in the expression of Tnap. Using EDS technology we confirmed an increase in calcium (Ca) and phosphorus (P) content in UAC cartilage vs their age-matched controls, beginning at 2 weeks post-operation. Only one of four joints tested in 12- and 20-week UAC group was noticed an isolated mineral which was located closing to the deep zonal mineral deposition. They were rich in calcium and phosphorus, but had a low carbon content. Their averaged Ca/P ratio was 1.42 (ranging from 1.38 to 1.48) and 1.44 (range from 1.39 to 1.49) at 12- and 20-weeks UAC group, respectively, closely resemble to those of octacalcium phosphate (Ca/P=1.33) and tricalcium phosphate (Ca/P=1.50), two subtypes of BCP crystals. We then exposed ATDC5 cells to the synthetic BCP crystals. We found that BCP crystals (50μg/ml) reduced Col-II, Col-X and aggrecan expression, but upregulated the expression of Adamts5, Mmp3 and Mmp9, however, only transiently stimulated Tnf-α and Il-1β expression.
Conclusions: The present data indicated that mechanical stimuli by UAC enhance mineral accumulation of BCP-like minerals in the cartilage matrix of TMJ condyle at deep layer. The UAC induced aberrant mineralization deposition of BCP-like minerals can stimulate matrix degradation via promoting cartilage-degrading enzymes, and may contribute, at least in part, to the thinning of articular cartilage by expansion of the calcified cartilage, facilitate osteoarthritis progression.

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THE EFFECTS OF REHABILITATION PROGRAM AFTER TOTAL KNEE REPLACEMENT ON BALANCE AND FUNCTIONALITY

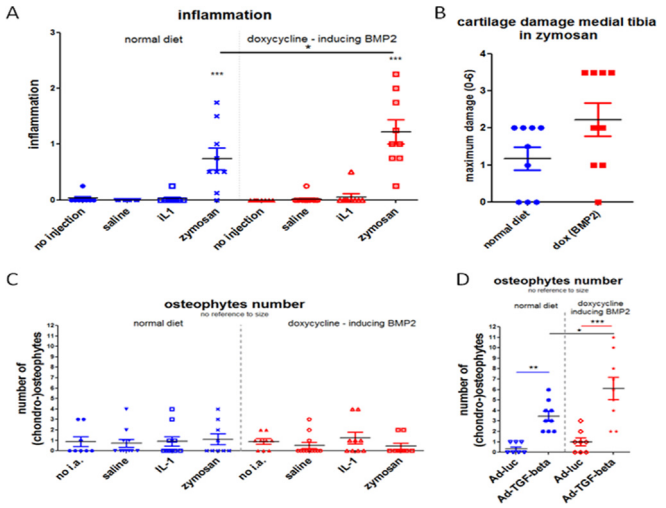
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Purpose: The purpose of the study was to evaluate the effects of the rehabilitation program after simultaneously total knee replacement on balance and functionality in patients with osteoarthritis and to compare the results with the healthy individuals.
Methods: Two study groups of osteoarthritic patients between ages 50-85 who underwent bilateral TKA and one control group (Group 3, n=20) were included in the study. Exercise program with frequent physical therapist supervision (2 session/1 week) was performed in Group 1 (n=20) and less supervised exercise program was performed Group 2 (n=20) (1 session/2 week). Exercise program was continued during the first month. All the patients were evaluated preoperatively as well as 1.and 2.month postoperatively. The patients were checked every week by telephone interview. Functionality was evaluated with WOMAC (The Western Ontario and McMaster Universities Arthritis) Index, dynamic balance with Timed Up&Go (TUG) and stair climbing test (SCT), static balance with single limb standing test (SLST).
Results: There was no statisitical significance between groups in terms of age (p=0,463). In preop evaluation there were significant differences between groups in all tests in favor of the control group. WOMAC pain scores in Groups 1 and 2 were similar in 1.month postop evaluation while the decrease in the pain score in Group 1 was higher than the scores of Group 2 and was close to the scores of healthy controls (p=0,00).
WOMAC stiffness scores were higher in postop period than preop period in both Group 1 and 2. The stiffness scores of Group 1 were significantly lower than Group 2 in 2. month postoperatively and were close to healthy controls (p=0,00).
WOMAC functionality scores of Group 1 and 2 were significantly higher in 1.and 2.month postop compared to preop period. The functionality scores of Group 1 were significantly higher in postop 2.month compared to Group 2 and were closer to the scores of healthy controls (p=0,00).
The completion time of TUG test of the groups were shorter in 1.and 2.month scores and were similar to the 1.and 2.month scores of controls (p=0,33).
SCT time of the groups were shorter in both 1.and 2. month postop period compared to preop period. Stair climbing time of Group 1 was significantly shorter than Group 2 in 2.month postoperatively and was closer to the scores of healthy controls (p=0,00).
SLST for both legs of Group 1 were significantly higher than Group 2 in 1. and 2. month postoperatively and were closer to healthy controls (p=0,00).
Conclusions: The patients which have applied exercise program after simultaneously bilateral TDR under the supervision of the physiotherapist 2 days a week for 1 month has obtained improvements in balance and functional parameters and it has been shown that their performances approach the healthy control group values.

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INFLAMMATORY FACTORS DO NOT ENABLE BMP2-INDUCED OSTEOPHYTE FORMATION

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Purpose: The main hallmarks of osteoarthritis (OA) are cartilage damage and osteophyte formation. The latter can clearly be observed in X-rays of patients. What causes these osteophytes in OA is not entirely clear. We have previously identified that in experimental models both TGF-beta and BMP2 can induce de novo osteophyte formation. In addition, when blocking TGF-beta signalling during experimental OA we were able to prevent osteophyte formation. Recently we have shown that chondrocyte-specific overexpression of BMP2 in a mouse model does not induce osteophyte formation on its own, but that it greatly enhances experimental OA-induced osteophyte formation. The latter could be blocked by inhibition of TGF-beta activity. Apparently, TGF-beta is involved in osteophyte formation but since there are inflammatory factors in (experimental) OA, it is still unclear whether they might play a role in osteophyte formation. This led to our question: can inflammatory factors enable BMP2 to induce osteophyte formation in a mechanically stable joint?
Methods: We exposed Col2a1-rtTA-BMP2 mice (inducible BMP2 with doxycycline) to a simple inflammatory trigger that has been implicated in OA: interleukin-1, and to a more complex inflammatory trigger: zymosan by intra-articular injection. Saline injections were used as controls. In addition, since we have previously identified TGF-beta as a major trigger for osteophyte formation in OA, we included a low-dose of adenovirus overexpressing TGF-beta (ad-TGF-beta) as a trigger, which on its own results in fibrosis but not yet osteophytes. We added ad-Luc as a viral control. Mice were either fed standard diet (non-dox) or a diet with doxycycline (dox) starting 7 days prior to i.a. injection for the entire duration of the experiment and 14 days after intra-articular injection of the adenovirus we isolated knee joints for histology.
Results: Saline injections combined with BMP2 did not lead to osteophyte formation. Moreover, neither did IL-1 injection combined with BMP2. Zymosan injection resulted in clear synovial inflammation, which was further enhanced by BMP2 overexpression. (fig 1A) Also, there were minor fissures in medial tibial cartilage in zymosan-injected joints, which showed a trend to becoming slightly more severe when combined with BMP2, but this was not significant. (fig 1B) Despite the overt inflammation induced by injection of zymosan, the combination of zymosan with BMP2 overexpression was insufficient to induce osteophytes. (fig 1C) Injection of adenoviral TGF-beta resulted in massive fibrosis and minor osteophyte formation. As expected, combining TGF-beta with BMP2 resulted in clearly enhanced osteophyte formation. (fig 1D)



Conclusions: Together these data show that neither a simple inflammatory trigger like IL-1, nor a more complex inflammatory trigger like zymosan enable BMP2 to induce osteophytes. In contrast, BMP-2 in combination with TGF-beta is a results in massive osteophyte formation. The latter confirmed our prior data where we showed that by blocking TGF-beta during experimental OA we could prevent BMP2 from inducing osteophytes.

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CARTILAGE GENE EXPRESSION CHANGES IN EARLY MOUSE DMM ARE DETECTED IN LATE HUMAN OA

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Purpose: The DMM mouse has become the model of choice for much experimental OA research because of its reproducible and relatively rapid disease onset, coupled to the mouse's genetic tractability. Nevertheless, the DMM model represents a mechanically induced form of OA developing quickly (2–8 weeks) in a young (10–12 week) animal whereas most human OA is in an ageing population with idiopathic and long term disease. We therefore compared the differentially regulated gene profiles accompanying damage in human OA cartilage with samples of cartilage progressing to damage taken from DMM mice.
Methods: Global gene expression was determined by RNAseq analysis on i) paired intact and damaged OA cartilage from 8 patients at TKR; and ii) mouse tibial medial compartment cartilage laser dissected from DMM and sham operated mice 2 weeks after operation. The expression