on the doxorubicin-induced activation of ICl,vol in rabbit articular chondrocytes using whole-cell patch-clamp technique.
Methods and Materials: Rabbit cartilages were collected from joints of male animals weighing 2.5 to 3.0 kg. The cartilage was dissected into slices and cultured in DMEM for 1-3 days. On the day of experiments, chondrocytes were isolated by enzymatic digestion. Whole-cell membrane current was recorded under conditions where Na+, K+ and Ca2+ currents were minimized. Real-time change in cell size was monitored using a CCD digital camera and the cross-sectional area of cell image was measured.
Results: Exposure of isolated chondrocytes to doxorubicin (1μM) resulted in an obvious increase in the membrane Cl- conductance without any appreciable change in cell size. The doxorubicin-evoked Cl- current exhibited many properties almost identical with ICl,vol phenotype, including outward rectification, prominent inactivation at large positive potential, inhibition by hyperosmotic cell shrinkage, and sensitivity to ICl,vol blockers, arachidonic acid or DCPIB . Pretreatment of cells with 17β-estradiol inhibited the ICl,vol activation by doxorubicin as well as subsequent apoptotic events such as AVD and elevation of caspase 3 activity. It was unlikely that 17β-estradiol produced a direct action on ICl,vol, because it had little effect on ICl,vol activated by hyposmotic cell swelling. On the other hand, the effect of 17β-estradiol was significantly attenuated by an estrogen receptor blocker.
Conclusions: These results suggested that 17β-estradiol may prevent the doxorubicin-induced apoptosis by interfering the activation of ICl,vol in rabbit articular chondrocytes.

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Chondrocyte micro-aggregates enhances neo-cartilage formation

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Purpose: We recently developed a novel injectable in situ crosslinkable Dextran-Tyramine 14kDa-DS=15 (Dex-TA) hydrogel, which has shown high potential for cartilage regeneration. However, cartilage repair strategies based on autologous chondrocyte implantation still rely on in vitro expansion to obtain sufficient cells with all inherent drawbacks such as dedifferentiation. Cartilage repair using mixtures of a limited number of chondrocytes with Mesenchymal Stem Cells (MSCs) can potentially overcome this hurdle. It remains unclear whether the hydrogels should be seeded with single-cell suspension or with preformed micro-aggregates of defined size. To test, this we have seeded hydrogels with microaggregates of chondrocytes, MSCs or a mixture of both and compared their performance with single-cell seeded hydrogels.
Methods and Materials: High throughput formation of micro-aggregates of 50, 100 and 200 cells was achieved in micromolds. Micro-aggregates were prepared of chondrocytes, MSCs or a mixture of both. Morphology, stability and chondrogenic capacity was evaluated. Aggregates with the optimal cell density of 100 cells were incorporated into Dex-TA hydrogels, cultured in vitro and in vivo and compared to single-cell seeded hydrogels.
Results: Micro-aggregates were formed in a very controlled manner and successfully incorporated into Dex-TA hydrogels. Aggregates formed by 100 cells showed a superior balance between stability and gene expression profile, with higher collagen type2 and Aggrecan expression. After incorporation of micro-aggregates into Dex-TA, long term stability and survival was observed, as well as enhanced matrix production, when compared to single-cell seeded hydrogels. More cartilage was formed in micro-aggregates consisting of a 50%/50% mixture of chondrocytes and MSCs, compared to both micro-aggregates of pure cell populations and to single-cell mixtures.
Conclusions: We conclude that neocartilage formation is greatly improved by seeding hydrogels with micro-aggregates instead of single-cell suspensions. In addition, this system provided preliminary information about the effect of micro-cocultures, showing enhancement of neo-cartilage formation when using 50% chondrocytes mixed with 50% MSCs.

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The effects of non-steroidal anti-inflammatory drugs on chondrocytes

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Purpose: Osteoarthritis (OA) is a painful disease with degenerating cartilage matrix components. For OA treatment, non-steroidal anti-inflammatory drugs (NSAIDs) are the most frequently prescribed medications to relieve pain and reduce inflammation. The effects of NSAIDs on cartilage matrix remain controversial. To elucidate the effects of NSAIDs on OA treatment, we evaluated the effect of the selective cyclooxygenase-2 (COX2) inhibitors aceclofenac, celebrex and tiaprofenic acid, and non-selective COX inhibitor indomethacin on extracellular matrix of human chondrocytes in vitro.
Methods and Materials: Human chondrocytes were isolated from surgical specimen of OA patients. The chondrocytes were cultured in the normal medium (as a control group) or in the presence of aceclofenac, celecoxib, tiaprofenic acid and indomethacin for 7 days to examine the change in cell proliferation. The components of extracellular matrix of chondrocytes such as aggrecan and collagens were measured after 3-day treatment. The expressions of type I, II, and X collagens and aggrecan were determined by quantitative RT-PCR. The amount of total glycosaminoglycan (GAG) was measured by 1,9-dimethylmethylene blue (DMMB) binding.
Results: There is no significant difference in the cell proliferation rates in the presence or absence of NSAIDs for 7 days. The expressions of type I, II, and X collagens are reduced in the presence of the tested NSAIDs. However, the expression of aggrecan are significantly increased in the presence of aceclofenac, celecoxib and tiaprofenic acid. In addition, aceclofenac significantly increased total GAG production compared to those of celecoxib, indomethacin, tiaprofenic acid and control.
Conclusions: These results suggest that NSAIDs could affect the expression and production of the extracellular matrix of human chondrocytes, including increasing glycosaminoglycan and aggrecan, and decreasing collagens. Aceclofenac has the most prominent effect in increasing total GAG production. The effects of these changes in extracellular matrix components on physical property of chondrocytes need further evaluation.

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TGFβ-1 administration on serum free-expanded chondrocyte enhances the expression of osteogenic markers and induces SMADs transcript regulation: in-vitro and in-vivo studies.

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Purpose: Culturing articular chondrocytes is required to perform cartilage resurfacing in tissue engineering-based approaches the use of serum-free medium (SF) can opportunely mimic the physiological (avascular) autocrine environment. Previously data showed that TGFdministration during the expansion phase induces loss of matrix components, positive immunodetection for type-X Collagen and apoptosis once in 3D culture system and, after osteogenic induction, TGF-expanded cells strongly mineralized with respect to SF-expanded cells. Thus we evaluated if TGF&beta-1 administration influence the expression of osteogenic markers and SMADs, signalling proteins involved in the chondrogenic development and differentiation. Moreover we evaluated the behaviour of TGF-expanded cells in an in-vivo ectopic model.
Methods and Materials: Human articular chondrocytes were expanded in SF, with or without TGFβ-1. Subsequently cells were collected and seeded statically in hydroxyapatite (HA) scaffold and after 48h in osteogenic induction medium, implanted subcutaneously in nude mice for the following four weeks. Decalcified sections were assessed by mean of haematoxylin-eosin immunocitostaining. Microarray and Real time-PCR analysis were performed after the expansion phase.
Results: TGF-expanded cells displaying an increased transcripts levels for Osteopontin (OP; 4.76±2.24, [mean±SD]), Bone Sialoprotein (BSP; 2.76±0.03) and Collagenase-3 (MMP13; 6.25±3.18), while Osteocalcin (OC) wasn't influenced by TGFβ-1 (1.02±0.28 fold-increase). Moreover, microarray analysis showed that TGFβ-1 administration influenced the SMADs expression. Qualitative-PCR analysis confirmed that treated cells displayed increased level of SMAD-1 mRNA (1,77±0.26) and down-regulated SMAD-3 transcript (0.35±0.28). Interestingly, after four weeks in-vivo TGF-chondrocyte/HA implant showed nascent deposition of bone matrix and numerous blood-vessels.
Conclusions: TGFβ-1 directs chondrocytes to acquire a different phenotype, firstly regulating the expression of SMADs signalling pathways. These cells showed high level of OP, BSP and MMP-13 mRNA indicating the predisposition to undertake the endochondral ossification-like progression and in-vivo analysis seem to validate this hypothesis.