they exhibited increased histological scores and better microstructural morphology.
Conclusion: This idea takes advantage of the auto-resected ACL tissue, makes use of the ACL-derived stem cells with preferable self-renewal and high expression of tendon associated markers, and also avoids a second operation for cell seeding; which gives a new strategy for clinical ACL reconstruction.
http://dx.doi.org/10.1016/j.jot.2016.06.113

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THE PHENOTYPE AND EXTRACELLULAR MATRIX PRODUCTION OF CHONDROCYTES TRANSFECTED BY Ras-shRNA AND Sox9 IN 3D DYNAMIC CULTURE SYSTEM

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Introduction: Cartilage has a limited capability for regeneration. Thus, there is an imperative need to develop new strategies for cartilage tissue engineering amenable for clinical use. Chondrocyte-based tissue engineering has exerted a promising approach for cartilage regeneration. However, the occurrence of de-differentiated chondrocytes severely inhibits the success of cartilage repair in clinical practice. In a recent study, three-dimensional (3D) static and dynamic culture shows better maintenance of chondrocyte phenotype, compared with traditional 2D culture of chondrocytes. The effect of dynamic 3D culture on chondrocytes transfected by target genes has not been intensively investigated.
Subjects and Methods: In this study, we investigate the effects of 3D dynamic culture on chondrocytes transfected by lentivirus with Ras-shRNA and Sox9. The chondrocytes were derived from the cartilage of the knee joint, which was isolated from two-week-old Sprague-Dawley rats. Chondrocytes (passage 2) with and without transfection were cultivated *in vitro* in alginate columns. The chondrocytes in columns were cultured in medium containing 10% fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin in high-glucose Dulbecco's modified Eagle medium (DMEM). The columns were imbedded in 3D-printed thin-film materials. There were four groups included in the current study. Group A: The transfected chondrocytes with stretching stimulation; Group B: The non-transfected chondrocytes with stretching stimulation; Group C: The transfected chondrocytes without stretching stimulation; Group D: The non-transfected chondrocytes without stretching stimulation. The stretching was performed at a specific frequency for two hours every day. After five days and 35 days of cultivation the samples were harvested for testing. The expression of Sox9, collagen type II (Col II), and glycosaminoglycans (GAG) was analysed using RT-qPCR and Western blot at the mRNA and protein level, respectively. The samples were also embedded and sectioned for H.E., Alcian blue, and immunohistochemical staining.
Results: Cells in group A showed more normal cartilaginous phenotype with abundant production of Col II, Sox9 expression, and GAG in comparison with those of group B. Group C showed a similar trend. The expression of cartilage specific genes was much higher than that of group D. Furthermore, the phenotype and collagen production of group C was higher than that of group B, which indicated that target gene transfection was more efficient to keep the cartilaginous phenotype and inhibit dedifferentiation. Not surprisingly, cells in group D (without transfection and stretching) performed poorest to inhibit dedifferentiation.
Discussion and Conclusion: In conclusion, 3D dynamic culture was more beneficial to keep the chondrocyte phenotype, in comparison with 3D static culture. Chondrocytes transfected by lentivirus with Ras-shRNA and Sox9 could efficiently produce an ECM. The combination of 3D dynamic culture and gene transfection could provide promising results to inhibit dedifferentiation of chondrocytes during the cell expansion process.
http://dx.doi.org/10.1016/j.jot.2016.06.114

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PDGF PLAYS A CRITICAL ROLE IN OA PROGRESS BY STIMULATING THE MIGRATION AND PROLIFERATION OF OSTEOCHONDROPROGENITORS

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Objective: To isolate and identify osteochondroprogenitors (OCPs) from articular cartilage of patients with osteoarthritis (OA) by using CD146 and study the relationship between the change of $CD146^+$ OCPs' biological function and the severity of OA. Finally, we try to verify the molecular mechanism that regulates the function of $CD146^+$ OCPs in the OA micro-environment.
Methods: Firstly, we collected surgical waste including articular cartilage and subchondral bone from patients undergoing total knee arthroplasty (TKA) during October 2013and July 2014 in our department. All the patients had much more severe cartilage damage in the medial femoral condyle than that in the lateral part. Both the lateral and medial condyles were proposed for digestion with 0.2% type II collagenase and for the isolation of $CD146^+$ OCPs. We did immunohistochemistry to detect the location of $CD146^+$ OCPs in OA cartilage and subchondral bone and compared the number, potential of proliferation, multilinage differentiation, and migration of $CD146^+$ OCPs between the lateral and medial femoral condyles. Secondly, we respectively isolated Nestin-Cre mesenchymal stem cells (Nes-Cre MSCs) and Osterix-Cre osteochondroprogenitors (Osx-Cre OCPs) from $PLCg_1$conditional knocked out ($PLCg_1$ KO) or wild type ($PLCg_1$Wt) mice of Nestin-Cre/R26-$PLCg_1$-loxP-YFP and Osterix-Cre/R26-$PLCg_1$-loxP-YFP by using Cre/loxp system. We compared the capacity of colony forming units (CFU) and multi-lineage differentiation of Nes-Cre MSCs and Osx-Cre OCPs between the KO and Wt mice. At last, we detected the potential of proliferation, differentiation, and migration of $CD146^+$ OCPs, Nes-Cre MSCs, and Osx-Cre OCPs of $PLCg_1$KO or $PLCg_1$Wt mice with or without the stimulation of PDGF-BB.
Results: $CD146^+$ OCPs were mainly located around the capillaries which invaded OA cartilage from subchondral bone and the number of $CD146^+$ OCPs was much higher in the medial femoral condyle than that in the lateral part. However, there was no significant difference of the potential of proliferation, differentiation, and migration of $CD146^+$ OCPs between these two parts. Nevertheless, compared to the capability of multi-lineage differentiation of MSCs, the potential of osteogenic differentiation of $CD146^+$ OCPs was higher, while the potential of adipogenic differentiation was lower, and potential of chondrogenic differentiation was similar. It was observed that the CFU of Nes-Cre MSCs and Osx-Cre OCPs of $PLCg_1$ KO mice was lower than that of $PLCg_1$Wt mice, while no significant difference of capacity of proliferation and multi-lineage differentiation existed between Nes-Cre MSCs and Osx-Cre OCPs of $PLCg_1$KO and $PLCg_1$Wt mice. The results showed that the ability of proliferation and migration of $CD146^+$ OCPs, Nes-Cre MSCs, and Osx-Cre OCPs of $PLCg_1$Wt mice dramatically increased after stimulated with PDGF-BB, while no substantial change of their multi-lineage differentiation potential existed before and after the stimulation of PDGF-BB. However, for the Nes-Cre MSCs and Osx-Cre OCPs of $PLCg_1$ KO mice, there was no obvious difference of the potential of proliferation, migration, and multi-lineage differentiation between the stimulation with PDGF-BB and without the stimulation of PDGF-BB.
Conclusion: PDGF/PDGFR/$PLCg_1$ signalling could regulate the proliferation and migration of OCPs *in vitro* in the OA microenvironment, which might play a role in the progression of OA.
http://dx.doi.org/10.1016/j.jot.2016.06.115

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INHIBITION OF YAP ACTIVITY BY VERTEPORFIN RESCUED THE ECM DEGENERATION IN OSTEOARTHRITIS

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Objective: Osteoarthritis (OA) is the most widely occurring global orthopaedic disorder. Current drugs are applied for inflammation inhibition and pain release, but no drugs can effectively prevent or even slow down its pathological process. We hypothesised that controlled release of YAP inhibitor, verteporfin, can regulate the composition of articular chondrocyte ECM, thus preventing OA cartilage from degeneration.
Methods: AFM elastic mapping was performed on cryosections of human cartilage and YAP subcellular localization in normal and OA cartilage tissues was tested using immunohistochemical staining. Primary human articular chondrocytes were cultured for up to seven days both on Col I-modified PDMS substrates and tissue culture plastics. The RNA-sequence was used to find out the different gene expression profiles of chondrocytes cultured in PDMS and plastics. Real-time qPCR, IF, and Western blot were used to assess the chondrocyte to authenticate the regulatory mechanism. Chitosan microsphere vehicles and Chitosan-VP microspheres were fabricated to repair the knee joint osteoarthritis by articular injection.
Results: We found activation of YAP in both OA patients and experimental OA mice, corresponding to higher stiffness of ECM in OA cartilage as compared to normal tissue. RNA-sequence results revealed signatures that indicate inactivation of YAP/TAZ transcriptional regulators emerged as lower presentation in the set of genes regulated by PDMS substrate with cartilage-physiological stiffness. We further confirmed the mechanism that cytoskeleton disorganization maintains chondrocyte phenotype through YAP cytoplasmic retention in PDMS. As YAP and downstream genes were activated during OA development, we found the inhibition of YAP activity by small molecule verteporfin-chitosan microspheres attenuated OA progression.
Conclusion: These findings will help us to understand the role of YAP in mediating ECM stiffening in OA and shed light on the involved signalling pathway, thus providing some new knowledge and methods for OA treatment. Together, we proved that stiffening of the extracellular matrix (ECM) as well as activation of YAP happened during the pathological process of OA, indicating that YAP may

also be involved in the physiological and pathological process of articular cartilage.
http://dx.doi.org/10.1016/j.jot.2016.06.116

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PHASE I CLINICAL TRIAL OF INTRA-ARTICULAR INJECTION OF AUTOLOGOUS MESENCHYMAL STEM CELLS FOR THE TREATMENT OF WRIST CHONDRAL DEFECT

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Introduction: Wrist chondral defect is a common cause of persistent joint pain, which may lead to functional impairments including reduced range of motion and diminished grip strength, affecting working ability and quality of life. There are various reported surgical treatment regimens but their effectiveness remains controversial due to the inherently poor regeneration ability of cartilage. Bone marrow-derived mesenchymal stem cells (MSC) are reported extensively to promote the regeneration of articular cartilage in various chondral defect and osteoarthritis animal models. While translation studies are on-going in knee OA, it is of clinical interest to explore the potential effectiveness of MSC therapy for the treatment of wrist chondral defect. As the first local MSC trial for chondral defect, we aimed to examine the feasibility and the safety, and to obtain data for sample size estimation for future study.
Subjects and methods: In this phase I single-arm trial, 10 patients (18 to 75 years old) with persistent post-traumatic chronic wrist pain, with imaging and previous arthroscopic evidence of wrist carpal bone chondral defects who opted for active treatment were invited to join (CREC Ref No. 2014.291-T). After arthroscopic washout and debridement, 10mL bone marrow was aspirated and subjected to the isolation and expansion of MSC cells in a certified clean room (ISO class 7). The MSC was characterized with a colony forming unit (CFU) assay, surface phenotypes (CD45, CD14, CD19, CD34, CD73, CD105, CD44, CD29, CD90, and HLA-DR), and a multipotent differentiation assay according to the International Society for Cellular Therapy guidelines. One month after bone marrow aspiration, characterised autologous MSCs (1 million cell per mL saline) with viability over 90% and clean from microbiological tests were injected back into the wrist joint of the patient. One-year follow-up assessments including functional wrist performance score and pain score, and other secondary assessments were carried out by trained personnel. The spread of data was tested for normality. Changes from baseline (pre-op) in all the measurements were determined with a t-test or Wilcoxon sign rank test where appropriate. Missing data was replaced with imputation under a missing-at-random assumption. Differences were considered statistically significant when $p < 0.05$ (SPSS V19).
Results: Six patients (5 male and 1 female), mean age of 38.5 years old fulfilling the inclusion and exclusion criteria were recruited during the reported period (January to December 2015). All procedures, including bone marrow aspiration, arthroscopic debridement, and intra-articular MSC injection, were uneventful and there were no signs of infection and nil complications noted or reported. It was practical to expand MSCs *in vitro* to a sufficient number for characterisation and injection in one month. Till now, six-month follow up data indicated the potential therapeutic effect of intra-articular MSC injection at single dose, as shown by numerical improvement in performance score and pain score.
Discussion and conclusion: This pilot clinical trial shows the safety and potential therapeutic effect of single dose autologous bone marrow-derived MSCs on persistent wrist chondral defect. Additional data from the second phase follow-up will provide more insight into the treatment of wrist chondral defect with MSCs.
http://dx.doi.org/10.1016/j.jot.2016.06.117

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OPTIMISATION OF CULTURE CONDITIONS FOR MAINTAINING PORCINE INDUCED PLURIPOTENT STEM CELLS

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Ground state porcine induced pluripotent stem cells (piPSCs), which retain the potential to generate chimeric animal and germline transmission, are difficult to produce. This study investigated morphological and biological progression at the early stage of porcine somatic cell reprogramming and explored suitable conditions to increase the induction efficiency of piPSCs. A cocktail of defined transcription factors was used to generate piPSCs. The amphotropic retrovirus, which carried human OCT4 (O), SOX2 (S), KLF4 (K), C-MYC (M), TERT (T), and GFP were used to infect porcine embryonic fibroblasts (PEFs). The number of clones derived from OSKM (4F) and OSKMT (4F + T) was significantly higher than that from SKM (3F) and SKMT (3F + T), suggesting that OCT4 played a critical role in regulating porcine cell reprogramming. The number of alkaline phosphatase positive clones from a medium with leukaemia inhibitory factor (LIF) and basic fibroblast growth factor (bFGF) (M1 medium) was significantly higher than that with insulin and 2i PD0325901/CHIR99021 (M2 medium), indicating that insulin and 2i could not effectively maintain piPSC propagation. In the M1 medium, piPSC lines could not maintain the typical self-renewal morphology on gelatin-coated and Matrigel-coated plates. Without the mouse embryonic fibroblast (MEF) feeder, piPSCs started to simultaneously differentiate. Based on the potential for self-renewal and activation of pluripotent markers, we found that the culture condition of 4F + T plus LIF and bFGF plus MEF feeder promoted PEF reprogramming more efficiently than the other conditions tested. Two piPSC lines (IB-1 and IB-2) were derived and maintained for up to 20 passages *in vitro*.
http://dx.doi.org/10.1016/j.jot.2016.06.118

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INJECTABLE AND ROBUST BIOPOLYMER-BASED SUPRAMOLECULAR HYDROGELS FOR REGENERATIVE MEDICINE

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Osteoarthritis (OA), which is symptomised as progressive degradation of articular cartilage in human diarthrodial joints, has become one of most prevalent, debilitating diseases in modern society. To address the increasing clinical demand for more effective treatment of OA, significant progress has been made in biotechnology, especially in the field of biomaterials. In the most recent decade, increasing research emphasis has been placed on the "bio" part of biomaterials. In our lab, we have shown that functionalisation of the hydrogels with biomimetic peptides promotes the differentiation of the hMSCs. In addition to the biofunctionalisation, the physical functions of the biomaterials are also critical to the successful translation of biomaterials to clinical treatment of cartilage diseases. Although biopolymer-based chemical hydrogels, with biopolymers covalently crosslinked, have been widely used as scaffolds for tissue engineering due to good stability, their permanent network structures and brittleness limit their applications in repairing load-bearing tissues, such as cartilage. In contrast, biopolymer-based supramolecular hydrogels, which are usually formed via self-assembly of physically interacting biopolymers are usually weak, as shown in "inverted vials", instead of freestanding 3D constructs and they are less stable than chemical hydrogels. Herein, we describe a novel host-guest macromer (HGM) approach for preparation of biopolymer-based freestanding supramolecular hydrogels. We have developed a series of injectable hydrogels with unique properties such as resilient mechanical property, bioadhesiveness, injectability, and promoting recruitment of endogenous cells that are desirable for potential clinical applications in the regeneration of soft musculoskeletal tissues such as cartilage, meniscus, and intervertebral discs.
http://dx.doi.org/10.1016/j.jot.2016.06.119

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CO-CULTURE OF HUMAN SYNOVIUM-DERIVED STEM CELLS AND CHONDROCYTES REDUCES HYPERTROPHY

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Introduction: Mesenchymal stem cells (MSCs) have emerged as a clinically relevant cell source for regenerative medicine, especially for cartilage repair; however, it still remains a challenge to recapitulate the functional properties of native articular cartilage using only MSCs. *In vitro* expansion of chondrocytes causes dedifferentiation. Lately, co-cultures of chondrocytes and bone marrow MSCs demonstrated enhanced functional properties of engineered cartilage. In this study, we aimed to assess the effect of co-culture of synovium-derived stem cells (SDSCs) and chondrocytes on *in vitro* chondrogenesis in serum-free TGF-β supplemented medium.
Methods: Isolation and expansion of cells: Human SDSCs and chondrocytes were isolated by sequential digestion from explants of total knee arthroplasty patients and incubated at 37 °C, 95 % humidity, and 5 % CO_2 in standard culture medium. We used passage 2 cells hereafter.
In vitro differentiation of cells: Expanded chondrocytes, SDSCs, and chondrocyte/SDSCs (5×10^5cells per pellet; co-culture ratio, 1:1) were cultured in chondrogenic medium for 1, 7, 14, and 21 days. The SDSC and chondrocyte/SDSC mixed pellets were cultured with the supplementation of 10 ng/mL of TGF-β1 and the chondrocyte pellets were cultured in chondrogenic medium only. The medium was changed twice per week.