of protein in a prolonged period. The composite scaffold could induce the adhesion and osteogenic differentiation of MSCs.
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LiCl PROMOTES CHONDROGENIC DIFFERENTIATION OF BMSCs IN INFLAMMATORY CONDITIONS INDUCED BY IL-1 THROUGH SUPPRESSING NF-κB SIGNAL PATHWAY

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Introduction: It is a complex process to regulate bone marrow mesenchymal stem cells' (BMSCs) chondrogenic differentiation, especially in inflammatory conditions. This study aimed to investigate the effect of LiCl in chondrogenic differentiation of BMSCs in inflammatory conditions and the possible mechanism in this process.
Subjects and Methods: BMSCs were treated with IL-1 in the process of chondrogenic differentiation. Along with IL-1, one group was treated with 10 nM LiCl and the other group with 10 nM GSK-3β. For each group, the glycosaminoglycan (GAG) amount was quantitatively tested and the mRNA of Sox 9, Collagen 2a, and Aggrecan were tested by RT-qPCR. The total NF-κB protein, p-NF-κB protein, and the NF-κB protein in cytosol or nucleus were tested by Western blot.
Results: Our results demonstrated that the index of chondrogenesis, such as the mRNA of Sox 9, Collagen 2a, Aggrecan, and the amount of GAG, were significantly decreased in the IL-1 group. However, in inflammatory conditions induced by IL-1, the indexes of chondrogenesis in LiCl group were significantly increased. Additionally, total NF-κB protein was increased, p-NF-κB protein was significantly decreased, and NF-κB protein in the nucleus was significantly decreased. All of these in GSK-3β group were just opposite to the LiCl group.
Discussion and Conclusion: These results strongly suggest that the ability of BMSCs' chondrogenic differentiation were decreased in inflammatory conditions induced by IL-1, but LiCl could enhance the ability although with IL-1. The possible mechanism was LiCl promotes the phosphorylation of the NF-κB protein and the transfer into nucleus, and then suppresses the NF-κB signalling pathway.
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DECELLULARISATION OF THE PORCINE TENDON-BONE INTERFACE FOR TISSUE ENGINEERING

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Background: High stress levels occur at interfaces between mechanically dissimilar materials. Therefore, fixation of soft tissue to bone, e.g. after bone tumour removal, has a high incidence of failure after surgical repair. Regenerating tendon/ligament-to-bone insertions, "entheses", by tissue engineering offers a promising solution to this challenge. Decellularisation of porcine Achilles' tendon entheses might elicit physiologically relevant scaffolds for tissue engineering. In this study, we establish a protocol to decellularise porcine entheses as potential scaffolds for interface tissue engineering.
Subjects and Methods: Achilles' tendons with attached calcaneus were harvested from six month old pigs and cut into 2x6x10mm^3 samples. Group 1 was chemically treated with a PBS solution containing 0.5% sodium dodecyl sulfate (SDS) and 1% triton100 (2s2t) for 48 hours, group 2 for 72 hours. In both groups, incubation was performed on a shaker at room temperature with exchange of detergents every 24 hours. A custom-made hydrostatic decellularisation device was used for group 3. Untreated/ PBS-treated samples were used as controls. Decellularisation was assessed histologically with HE staining and Masson staining. Cell counts were performed on randomised regions of interest (ROI = 200x200μm^2) in tendon, bone, and interface and subsequently averaged. According to the DNeasy Blood & Tissue protocol, DNA content was assayed after the decellularisation process. Biomechanical system (zwick i1120, Zwick/Roell, Germany; sensor type: KAF-Z, 2.0mV/V = 2.5KN, A.S.T.GmbH Dresden, Germany) was applied to test samples' mechanical characteristics, such as rupture load, stiffness, and Young's modulus. Statistical analysis was conducted using GraphPad Prism 6 software. Treatment groups were compared to evaluate decellularisation efficiency.
Discussion and Conclusion: Treatment of porcine Achilles' tendon entheses with 0.5% SDS + 1% Triton washing for 72 hours resulted in the most efficient and complete decellularisation. Structure and integrity of the enthesis matrix was preserved. Following decellularisation treatment of 2S2T 72 hours, there was a 98% reduction in local cells ($p<0.01$). While, all treated groups exhibited a statistically significant reduction in DNA versus the untreated group ($p<0.01$), no statistically significant difference was observed among all the treated groups of DNA remained radio. The biomechanical tests indicated that, samples throughout the process had similar mechanical characteristic of rupture loads, stiffness, and Young's modulus to the untreated group. In the future, the decellularized scaffolds will be recellularised with mesenchymal stem cells to investigate matrix effects on differentiation of cells as well as developing scaffolds for enthesis tissue engineering.
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SCREENING KEY FACTOR FROM SENSORY NERVES REGULATING ON MACROPHAGE IN PROMOTING BONE DEFECT HEALING AND ITS MECHANISM

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To investigate the mechanism of bone defect healing is of great significance for taking corresponding measures to promote the bone defect healing. Macrophages, as one of the essential factors in the process of bone defect healing, can promote bone formation and inhibit bone absorption. However, the factors regulating macrophages during bone repair is unknown. Nerves can manipulate the balance of the differentiation of bone cells and macrophages and can also promote bone repair. In the previous study, it was found that sensory nerves can promote bone defect healing significantly, while motor nerves did not have an evident role. In this study, we used iTRAQ to compare the differential expression proteins in sensory and motor nerves and anticipate obtaining the functional neuropeptide regulating macrophages. We found 19 factors' expression in sensory nerves significantly higher than in the sciatic nerve. By using a literature review, we focused on MIF (macrophagemigration inhibitory factor), which can affect migration and phagocytosis of macrophages and is also closely related to bone repair. We further used a rat tibia drilling model as a subject to detect the distribution of MIF during bone repair. We found MIF abundantly distributed in the lysosomes of macrophages, showing macrophages englobe MIF; the macrophages comprising MIF were distributed in the bone repair active region, such as the bone remodelling region around the trabecular and blood vessels. The cells are abundant in the primary callus stage than the mature callus stage. These results indicate that MIF is involved in the regulation of macrophages in promoting bone defect healing, and the regulation may be through endocytosis of macrophages.
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AMECM/DCB SCAFFOLD PROMPTS SUCCESSFUL TOTAL MENISCUS REGENERATION IN A RABBIT TOTAL MENISCECTOMY MODEL

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Objective: The meniscus lacks self-repair ability because of its unique structural features, so the treatment of meniscus lesions is a big challenge all the time. The aim of this study is to construct a composite meniscus scaffold (AMECM/DCB scaffold) with an acellular meniscus extracellular matrix (AMECM) and decalcified cancellous bone (DCB), test the physicochemical characteristics *in vitro* and immunological properties *in vivo*, implant the scaffolds in New Zealand rabbits that underwent total meniscectomy, and evaluate the meniscus regeneration and articular cartilage protective effect.
Methods: We utilised an acellular meniscus extracellular matrix (AMECM) and demineralized cancellous bone (DCB) to construct three different kinds of three-dimensional porous meniscus scaffolds (AMECM scaffold, DCB scaffold, and AMECM/ DCB scaffold). We detected the physicochemical characteristics of the three different scaffolds, including micro-structure analysis through SEM (scanning electron microscope), the scaffold composition detection through histological and biochemical analysis, and mechanical property testing with BOSE mechanical testing machine. We implanted the three different scaffolds subcutaneously in the rats to assess the immunological rejection of the scaffolds. We then seeded the meniscus fibrochondrocytes into the scaffolds and then observed the microstructure with SEM, tested the cytotoxicity of scaffolds through live/dead cell staining, and detected the glycosaminoglycan (GAG) and collagen content secreted by the fibrochondrocytes in the three scaffolds after 3, 7, and 14 days. We implanted the three different scaffolds into New Zealand rabbits which underwent total meniscectomy and then evaluated the meniscus regeneration and cartilage protective effect of the three different groups through macroscopic observation, histological analysis, X-ray, MRI, biomechanics tests, and RT-PCR at three and six months.
Results: The SEM results showed that all the three different scaffolds possessed a three-dimensional porous structure and good porosity. The GAG content of AMECM/DCB scaffold and AMECM scaffold was higher than that of the DCB scaffold. The biomechanical property of AMECM/DCB scaffold was superior to those of the

other two scaffolds (compressive modulus, tensile modulus). No significant immunoreaction was observed in the rabbits for all three different scaffolds. The rabbit meniscus fibrochondrocytes can grow well in all the three scaffolds and the live/dead cell staining result showed that there were no significant difference in the three scaffolds; however, the fibrochondrocytes seeded in the AMECM/DCB scaffold and the AMECM scaffold could secrete more GAG and collagen than the DCB scaffold. The *in vivo* repair experiment showed that, there was no significant meniscus regeneration in the control group and the AMECM group, and just a little synovium growth-in. The neo-meniscus appeared in the AMECM/DCB group and the DCB group and the semi-quantitative histologic score and Ishida score results showed that the neo-meniscus in the AMECM/DCB group were better than those of the DCB group. The Mankin scores of the relevant femur condyles cartilage and the tibial plateau cartilage in the AMECM/DCB group was higher than those in the other two groups. Both the X-ray result (K-L grade score) and MRI result (WORMS score) showed that the repair effect of the AMECM/DCB group was better than those of the other two groups. The tensile modulus of the neo-meniscus at three months was higher than that of six month groups, while at the same time point, the tensile modulus of the neo-meniscus in the AMECM/DCB group was higher than that of the DCB group. The RT-PCR result showed that aggrecan, Sox9, and collagen II expression at three months is higher than those of six month groups, while at the same time point, aggrecan, Sox9, and collagen II expression of neo-meniscus of the AMECM/DCB group was higher than that of the DCB group.

Conclusion: The AMECM/DCB scaffold has a good three-dimensional porous structure, better biomechanical property compared to the AMECM scaffold and the DCB scaffold, and can promote GAG and collagen secretion of fibrochondrocytes. The *in vivo* repair experiment results indicated that the AMECM/DCB scaffold could promote meniscus regeneration and restrain osteoarthritis (OA) progress. The AMECM/DCB scaffold is a good choice for meniscus tissue engineering.

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THE INHIBITIVE EFFECTS OF SOX-9 ON THE DEDIFFERENTIATION OF CHONDROCYTES UNDER VARIOUS CULTURE CONDITIONS

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Introduction: Sox-9 plays an important role in the formation and degeneration of cartilage. It can affect the differentiation, dedifferentiation, and even redifferentiation of chondrocytes. The objective of this study is to investigate the effects of Sox-9 on the dedifferentiation and chondrogenesis of chondrocytes under two-dimensional (2-D) and three-dimensional (3-D) culture conditions.

Materials and Methods: 1. Chondrocytes were obtained from the distal femoral cartilage of knee joints isolated from two-week-old Sprague-Dawley rats. After cell expansion *in vitro*, the cells of passage 2 (P2) were used for further study.

2. Two different culture conditions were investigated in this study. Group A: 2-D culture (conventional plate culture); Group B: 3-D culture (pellet culture). Each group was further divided into two sub-groups: Group A1, Group A2; Group B1, Group B2. In Group A1 and B1, the cultured cells were chondrocytes transfected with lentiviral vector-mediated Sox-9. The non-transfected chondrocytes cultured in Group A2 and B2 were regarded as control groups.

3. In group A1 and A2, cells were proliferated in flasks and sub-cultured at 80% confluence. The cells of P2, P4, and P8 were collected for analysis. In group B1 and B2, the pellets were cultured and harvested for analysis at 2, 4, and 8 weeks after seeding. The transcription level of Sox-9 was evaluated by real-time quantitative (RT-PCR) assay. The cartilage-related specific genes including collagen II, IX, X, and GAG were examined by RT-PCR. The matrix production was examined by immunohistochemistry staining and a western blot test.

4. The data was compared among groups. All experiments were performed at least three times. Results are expressed as the mean $\pm$ SD. Paired t-tests were used for intergroup comparison and a p-value$<$0.05 was considered statistically significant.

Results: (1) The production of collagen and glycosaminoglycan decreased significantly with the expansion of non-transfected chondrocytes in 2-D culture. The immunohistochemistry staining and alcian blue staining also confirmed the reduced synthesis of cartilage ECM. In contrast, transfected cells showed rigorous proliferation and nearly normal phenotype. With cell expansion, the cartilage specific genes including Collagen II, IX, X and GAG showed increased production in mRNA and protein level. (2) Pellets of groups B1 and B2 showed the translucent, cartilage-like tissue. The volume and hardness of pellets continuously increased with culture time. After eight weeks of culture, the diameter of pellets approximately increased to 5 mm. The transfected chondrocytes were distributed uniformly in the pellet and had good viability. Furthermore, the collagen production and DNA content was much higher than those of the non-transfection group.

Discussion and conclusion: In conclusion, 3-D culture is beneficial for chondrogenesis and inhibits the dedifferentiation of chondrocytes after cell expansion. The cell transfected by lentiviral vector-Sox9 showed more intensily enhanced chondrogenesis in comparison with that of conventional pellet culture.

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HYPOXIA ENHANCES ENGINEERED CHONDROGENESIS THROUGH COORDINATING CHONDROCYTE GLUCOSE METABOLISM AND DIFFERENTIATION

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Background: Articular cartilage, a highly organized avascular connective tissue with substantial durability, has very limited capacity for self-repair following injury or degeneration. Chondrocytes are located in a hypoxic microenvironment during cartilage repair. Hypoxia inducible factor-1 alpha (HIF-1α) is identified as a key mediator for cellular adaptation to hypoxia. However, the molecular mechanisms of hypoxia regulation in chondrocytes' differentiation and metabolism remain to be defined.

Subjects and methods: Chondrocytes isolated from new-born mice were subjected to micro-mass or engineered 3D cultures under normoxia (21% O_2) or hypoxia (2% O_2). The chondrogenic marker genes' expression was examined by real-time PCR and protein expression was detected by Western blot and immunohistochemistry. Histochemical staining (Alcian blue or Safranin O staining) was performed to examine the cartilaginous extracellular matrix (ECM) formation. To investigate the effect of hypoxia on glucose metabolism, PCR array, real-time PCR, and Western blot analyses were performed to reveal the alterations of genes and protein expression in chondrocytes with or without HIF-1α deletion.

Results: In micro-mass culture, we found that hypoxia increased ECM proteoglycan synthesis after seven days culture, which was accompanied by up-regulation of chondrogenic marker genes' expression including SOX5, SOX9, Col2, and Aggrecan. Similar phenotypes were observed in the 3D culture system at days 7 and 14, in which the expression of the above chondrogenic marker genes was up-regulated and the intensity of Alcian blue and Safranin O staining was enhanced. Immunostaining showed an increase of HIF-1α, Glut1 and proliferating cell nuclear antigen (PCNA) positive cell numbers in 3D culture system, under hypoxia rather than that of normoxia. Real-time PCR and Western blot analyses showed up-regulation of HIF-1α, PHD2, and Glut1 levels, which was accompanied by increased glucose uptake of chondrocytes. Interestingly, 6-phosphofructokinase, liver type (PFKL) and PGK1 mRNA and protein were dramatically up-regulated under hypoxia rather than that of normoxia, revealed by PCR array analyses and Western blot, respectively. The promoted effect of hypoxia on the above protein expression was eliminated following deletion of HIF-1α in chondrocytes.

Discussion and conclusion: Our results indicate that hypoxia regulates key components of the glucose metabolism pathway through HIF-1α in chondrocytes and hypoxia enhances engineered chondrogenesis through coordinating glucose metabolism and chondrogenic differentiation.

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INTRA-ARTICULAR INJECTION OF ACL-DERIVED STEM CELLS COMBINED WITH SILK-COLLAGEN SCAFFOLD FOR ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION

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Objectives: This study aims to inject anterior cruciate ligament (ACL)-derived stem cells into the knee joint combined with Silk-Collagen scaffold for ACL reconstruction, which could enhance ACL regeneration.

Methodology: We established a rabbit ACL reconstruction model, implanted with a silk-collagen scaffold. The resected ACL tissue was used for ACL-derived stem cell isolation and culture, one week later they are intra-articularly injected. After three months and six months, the new ACL tissue was collected and evaluated.

Results: The silk-collagen scaffold combined with intra-articularly injected ACL-derived stem cells was found to enhance ACL regeneration. After six months,