213
THE EFFECT OF LOW INTENSITY PULSED ULTRASOUND TREATMENT COMBINED WITH MESENCHYMAL STROMAL CELL INJECTION FOR CARTILAGE REGENERATION IN A KNEE OSTEOCHONDRAL DEFECT MODEL OF RATS.

S. Yamaguchi †‡, T. Aoyama †, A. Ito †‡, M. Nagai †, H. Iijima †, J. Tajino †, X. Zhang †, W. Kiyan †, H. Kuroki †. † *Human Hlth. Sci.s, Graduate Sch. of Med., Kyoto Univ., Kyoto, Japan;* ‡ *Japan Society for the Promotion of Sci., Tokyo, Japan*

Purpose: Once cartilage is injured, it rarely recovers spontaneously, because of their poor intrinsic healing capacity. Cell transplantation therapy is anticipated to regenerate cartilage defect. Mesenchymal stromal cell (MSC) is one of expecting cell sources for cartilage repair due to their character including the capability which differentiate into chondrocyte. However there were few study that verified efficacy and safety of aftertreatment post cell transplantation. There were some reports that low intensity pulsed ultrasound (LIPUS), which is used for bone fracture treatment, could stimulate MSC differentiation into osteo-/chondro-cyte in vitro. The aim of this study was to investigate whether LIPUS treatment combined with cell therapy could affect cartilage regeneration for a knee osteochondral defect model of rats.
Methods: This study was approved by the animal research committee of our facility. An osteochondral defect of 1mm diameter was created on both femur grooves of twelve Wistar rats at 12-week old. Four weeks after creation of the defect, 1.0×10^6 allogeneic bone marrow MSCs diluted with phosphate-buffered saline (PBS) was transplanted into right knee joint by intra-articular injection and PBS without MSC was injected into left knee joint. The rats were divided into 2 interventions: without or with LIPUS treatment. Two days after injection, the rats with LIPUS were subjected to LIPUS treatment according to parameters borrowed from those for bone fracture treatment, 20 min/day, 5 days/week, to both knee joints. After 4 and 8 weeks intervention, the rats were euthanized, femora were removed and divided into four groups: Control group (PBS injection), LIPUS group (PBS injection with LIPUS treatment), MSC group (MSC injection) and MSCL group (MSC injection with LIPUS treatment). The 6-μm thick serial sections of the femur specimen stained with safranin-O and hematoxylin-eosin were examined and scored with Wakitani's cartilage repair score. The collagen type I and II expressions were also observed by immunohistochemical methods.
Results: Four weeks after intra-articular injection, the histological score were as follows, Control group: 8.7±2.36, LIPUS group: 4.7±1.31, MSC group: 4.7±1.31, MSCL group: 4.3±0.65. The defect area was filled with repair tissue which wasn't hyaline cartilage in Control group. Repair tissue in Control group was mostly expressed by collagen type I, but collagen type II expression was restricted in deep zone. In LIPUS, MSC and MSCL group, repair tissue mostly included hyaline cartilage like cell morphology, and showed SO staining intensity in middle zone. The repair tissue in these three group was thicker than Control group. The expression of collagen type II was observed in wide range of repair tissue in LIPUS, MSC and MSCL group, but the expression of type I collagen was observed through surface and middle zone in these groups. Eight weeks after intra-articular injection, the histological score were as follows, Control group: 7.7±2.36, LIPUS group: 7.0±1.96, MSC group: 4.7±1.31, MSCL group: 4.0±0.00. In Control and LIPUS group, fibroblast like cell morphology was observed and SO intensity was reduced. The expression of collagen type II was attenuated through surface to middle zone in Control group, while attenuated in surface zone of LIPUS group. In MSC and MSCL group, hyaline cartilage like cell morphology was observed in repair tissue, but the SO intensity was reduced. The expression of collagen type II was attenuated in surface zone of MSC and MSCL group. The expression of collagen type I was located in surface zone of all group or middle zone in some specimen.
Conclusions: It might be indicated LIPUS treatment or MSC injection could stimulate cartilage regeneration in 4 weeks after MSC injection, but repaired cartilage stimulated by LIPUS treatment was deteriorated in 8 weeks after MSC injection. In this experiment condition there might be little interactive effect between LIPUS and MSC injection for cartilage repair.

214
THE EFFECT OF SYSTEMIC ADMINISTRATION OF GRANULOCYTE-COLONY STIMULATING FACTOR (G-CSF) ON FULL THICKNESS CARTILAGE DEFECT IN A RABBIT

T. Sasaki, T. Sasho, S. Yamaguchi, Y. Akatsu, J. Katsuragi, T. Fukawa, J. Endo, H. Hoshi, Y. Yamamoto, K. Takahashi. *Chiba Univ. Graduate Sch. of Med., Chiba, Japan*

Purpose: Bone marrow stimulation is in use clinically as a treatment option for cartilage defects. Theoretically mesenchymal stem cells reside in bone marrow are induced into cartilage defects followed by cartilage repair with this technique. Although mostly good clinical results were reported, histology revealed repaired cartilage was fibro- to hyaline-like cartilage.
The aim of this study is to investigate whether systemic administration of G-CSF, stimulant of bone marrow, could improve the quality of repaired tissue using full thickness articular cartilage defect model of a rabbit.
Methods: Thirty 12week-old male New Zealand White rabbits were divided into three groups.
The low dose group (n=10) received daily 10μg/kg of G-CSF, the high dose group (n=10) 50μg/kg, subcutaneous injections for three days, prior to creating cartilage defects. To the control group (n=10), saline was administered for three days.
48 hours after the first injection, a 5.2mm diameter cylindrical osteochondral defect was created in the center of the femoral trochlea.
4, 12 weeks after the procedure, status of repaired tissue was evaluated by macroscopically as well as microscopically.
Results: Macroscopically, the defect fillings and the tissue qualities were better in G-CSF group than in the control group at 4 weeks. (High dose group showed better than the low dose group.)
Qualities of repaired cartilage were better in the G-CSF group at 12 weeks but the defect fillings were better in the control group, which assumed to be hypertrophy of fibrocartilage.
Conclusions: Macroscopically, G-CSF administration prior to bone marrow stimulation was effective in modifying quality of repaired tissue.

215
INTRODUCTION OF MESSENGER RNA INTO KNEE ARTICULAR CARTILAGES USING POLYPLEX NANOMICELLE

H. Aini †, K. Itaka ‡, U-i. Chung †, S. Ohba †. † *Dept. of Bioengineering, The Univ. of Tokyo Graduate Sch. of Engineering, Tokyo, Japan;* ‡ *Div. of Clinical Biotechnology, Ctr. for Disease Biology and Integrative Med., The Univ. of Tokyo Graduate Sch. of Med., Tokyo, Japan*

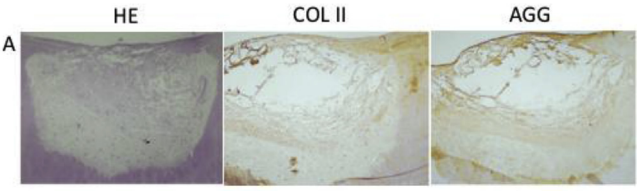
Purpose: Osteoarthritis (OA) is a degenerative joint disease that is caused by an imbalance in cartilage degeneration and synthesis, which results in pain and low quality of life in patients. It is a major health problem in elderly generation. Besides painkillers and anti-inflammatory drugs alleviating OA symptoms, disease-modifying osteoarthritis drugs (DMOADs) have drawn much attention. Given the fact that gene products stimulate chondrogenesis or break down cartilage matrix, delivery of therapeutic genes as DMOADs to the articular cartilage is a promising strategy for the treatment of OA. However, there are concerns regarding safety and efficiency on the introduction of nucleic acids in vivo. Although viral transduction shows high efficiency of gene introduction, its application is limited because of strong immunogenicity and toxicity. Plasmid DNA transfection has a risk of insertion into host genome. Messenger RNA (mRNA) introduction would directly induce the expression of therapeutic proteins in target cells without any risk of insertion mutagenesis. A biocompatible gene carrier based on self-assembly of a polyethylene glycol (PEG)-polyamino acid block copolymer, polyplex nanomicelle, was recently shown to achieve in vivo mRNA introduction by solving two major limitations for the in vivo mRNA delivery, instability and immunogenicity of mRNA. In this study, we examined the efficacy of the polyplex nanomicelle-mediated mRNA introduction into the articular cartilage of intact or surgically-induced OA knees in mice, aiming to apply this strategy to the treatment of OA

Methods: PEG-polyamino acid block copolymer was used as a carrier of mRNA. We injected 10 μl solution of polyplex nanomicelles carrying various amount of mRNA of luciferase or green fluorescent protein (GFP) into mouse knee joints. The luciferase mRNA expression was visualized at different time points by the IVIS imaging system. GFP expression was analyzed in histological sections. For mRNA introduction into OA cartilages, OA was induced by surgically transecting the medial collateral ligament and removing the medial meniscus in knee joints of 8-week old male C57BL/6 mice. A month after the surgery, the development of OA was confirmed by safranin-O staining and the OARSI scoring system. We then injected 10 μl solution of polyplex nanomicelles carrying mRNA of interest into OA knee joints every other day or once every 4 days for 2 weeks.
Results: In vivo imaging of luciferase activities revealed that intra-articular injections of polyplex nanomicelles carrying luciferase mRNA induced the production of functional luciferase proteins around the knee joints in mice. Luciferase activities were observed at 24 hours after injection and they were maintained for more than 4 days. When we injected polyplex nanomicelles carrying GFP into intact and surgically-induced OA knee joints in mice, GFP expression was observed not only in both intact and OA cartilages, but also in the meniscus. There was no symptom associated with immune reactions in the mRNA injected joints.
Conclusions: These results indicate that the polyplex nanomicelle successfully introduced exogenous mRNA into cells around joints, supporting the therapeutic potential of this strategy in the treatment of OA. mRNA introduction will enable us to utilize transcription factors for the treatment of degenerative diseases in disease stage-specific manners. Thus, this is an important initial step of the development of mRNA introduction-based therapeutic strategies for OA.

216
HUMAN CARTILAGE REPAIR USING HUMAN MESENCHYMAL STEM CELLS AND COLLAGEN SCAFFOLDS

C. Sanjurjo Rodríguez †, T. Hermida Gómez ‡, I. Fuentes Boquete †, F. De Toro Santos †, F. Blanco García ‡, S. Díaz Prado, Sr. †. † *Universidade da Coruña, A Coruña, Spain;* ‡ *Instituto de Investigación Biomédica de A Coruña, A Coruña, Spain*

Purpose: The purpose was to study chondrogenesis of adult human Bone Marrow Mesenchymal Stem Cells (hBMSCs) when culture on collagen (Col) biomaterials. Furthermore, we evaluated the usefulness of the generated chondrogenic constructs to repair cartilage in an *in vitro* lesion model.
Methods: hBMSCs were cultured during 30 days in chondrogenic medium, with Transforming growth factor β-3 (TGFβ-3), and in a control of DMEM medium on type I Col biomaterials. We have tested growth and cell morphology on the constructs by histochemical and Scanning (SEM) and Transmission (TEM) Electron Microscopy. Chondrogenic differentiation was evaluated in all the constructs by histochemical and immunohistochemistry analyses and molecular biology. 3 mm lesions were made in human cartilage biopsies and were pretreated with 10ng/ml IL-β for 24 hours. $2x10^5$ BMSCs were seeded on type I Col biomaterials, introduced inside the lesion, and cultured during 30 days in chondrogenic medium with TGFβ-3. Repaired tissue was evaluated by histochemistry and immunohistochemistry, and macroscopically using the ICRS scale.
Results: Histology showed that hBMSCs have grown through the scaffolds, being the cell percentage higher than 75% in the chondrogenic constructs. It was observed a big amount of extracellular matrix (ECM), which showed proteoglycan and type II Col positivity in chondrogenic but not in control constructs. By measurement of relative expression levels (REL), type II Col and aggrecan gene expression was seen only in cells grown in chondrogenic medium. Electron microscopy showed a big amount of mitochondria and oval/rounded shape cells in chondrogenic samples. In the *in vitro* model, histochemistry showed that after 30 days, the cells filled almost the 90% of the lesion with a cellular density in the graft higher than the native cartilage (Fig. 1A, H-E). We found integration of the neotissue with the border of the lesion. Regarding the ECM of the neotissue was a mixture of hyaline- and fibrocartilage, finding chondrocytes inside lacunas and an ECM positive for type II Col and aggrecan (Figure 1A, COL II and AGG). In the macroscopic ICRS assessment, the score of the repair was 21 out of 24 points (Fig. 1B).
Conclusions: Data showed that hBMSCs are able to grow on Col biomaterials and differentiate to a chondrocityc-like phenotype. These chondrogenic constructs are able to repair an *in vitro* human cartilage lesion after a 24-hour-pretreatment with 10ng/ml IL-β. *Acknowledgements*: Opocrin S.P.A.; CAM (S2009/MAT-1472); CIBER-BBN; Red Gallega de Terapia Celular, Xunta de Galicia (R2014/050); GPC, Xunta de Galicia (GPC2014/048); Fundación Española de Reumatología (2014 grant); Fundación Profesor Novoa Santos.



B

	30 days
Degree of defect repair	4 points (>90%)
Integration with the surrounding cartilage	4 points (Complete integration with surrounding cartilage)
Macroscopic appearance	3 points (Fibrillated surface)
Overall repair assessment	10 points (nearly normal)
Total (24 points)	21 points

Cell Signaling

217
VIPERIN; A NOVEL CHONDROGENIC REGULATOR

M.M. Steinbusch †, M.M. Caron †, F. Eckmann ‡, E. Lausch ‡, L.W. van Rhijn †, B. Zabel ‡, T.J. Welting †. † *Dept. of Orthopaedic Surgery, Lab. for Experimental Orthopaedics, Maastricht Univ. Med. Ctr., Maastricht, Netherlands;* ‡ *Ctr. for Paediatrics and Adolescent Med., Univ. of Freiburg, Freiburg, Germany*

Purpose: The ribonuclease mitochondrial RNA processing (RMRP) gene encodes the RNA component of a multi-protein-RNA complex called RNase MRP. Mutations in the RMRP gene cause Cartilage Hair Hypoplasia (CHH). The main phenotypic hallmark of CHH is dwarfism, presumably caused by impaired growth plate development. Our preliminary data indeed show that RMRP is involved in chondrogenic differentiation; however, it is unclear via which mechanism. Viperin is an antiviral protein, located in the endoplasmic reticulum (ER; location of protein synthesis and secretion), and its mRNA is a known substrate of RNase MRP cleavage activity. As such, viperin expression has shown to be increased in CHH patient cells and during knockdown (KD, RNAi) of RNase MRP subunits, including RMRP RNA itself. As the chondrogenic function of RMRP is unclear and it is not known how mutations in RMRP cause CHH, we hypothesize that viperin functions as a chondrogenic regulator downstream of RNase MRP.
Methods: To investigate the expression of viperin during chondrogenic differentiation in vivo, viperin was detected in day 1 mouse growth plates by immunohistochemistry. Expression of viperin mRNA and protein was determined by RT-qPCR / Western blot in chondrogenically differentiating ATDC5 cells in the presence or absence of 1 nM bone morphogenic protein (BMP)-2 or -7. KD of viperin expression was achieved via RNAi and protein secretion was measured upon transfection of a secretable CMV-Gaussia Luciferase.
Results: To investigate whether viperin plays a role in developing chondrocytes in vivo, day 1 mouse growth plates were stained for viperin. Profound viperin staining was detected in hypertrophic chondrocytes. However, viperin was also expressed in a subpopulation of proliferative chondrocytes. In these cells a perinuclear staining could be observed, which is in agreement with the localization of viperin in the ER. To determine the role of viperin expression in more detail, viperin gene expression dynamics were determined during chondrogenic differentiation of ATDC5 cells. Expression of viperin mRNA and protein was first detected at day 4 in