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. Kiyani †, H. Kuroki †, 1 Human HLth. Sci., Graduate Sch. of Med., Kyoto Univ., Kyoto, Japan; 2 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 large osteochondral defect model of rats.

Methods: This study was approved by the animal research committee of our facility. An osteochondral defect of 1 mm diameter was created on both femur grooves of twelve Wistar rats at 12-week old. Four weeks after creation of the defect, 1.0 × 10^7 allogeneic bone marrow MSCs diluted with phosphate-buffered saline (PBS) was transferred 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 before 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 specimens 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 I 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.