LOW-INTENSITY PULSED ULTRASOUND STIMULATION ENHANCES PRODUCTION OF TIMP-1 FROM THE NUCLEUS PULPOSUS CELLS AND MCP-1 FROM MACROPHAGE

Introduction
Intervertebral disc herniation is one of the most important medical and socio-economical problems in the modern world. Oftentimes surgical intervention is applied as a primary treatment. However, it is known that sequestrated hernias can be treated conservatively by spontaneous resorption. Although mechanism of resorption is not well understood, previous studies raise involvement of macrophage as one primary factor in this process. Low-intensity pulsed ultrasound (LIPUS) accelerates hernia resorption in animal organ culture model in rats with matrix metalloproteinase-3 (MMP-3) activation through TNF-alpha and monocytechomotactic protein-1 (MCP-1) pathways [1]. The purpose of the current study is to evaluate the effect of LIPUS stimulation on cytokine production by nucleus pulposus cells and macrophage in vitro.

Materials and Methods
All animal experiments were performed under approval by the institutional review board. Ten-week old male Sprague-Dawley rats (n=50, 400-500g). Macrophages were collected 2 days after injection of 3% thioglycolate phosphate buffer solution into the abdominal cavity. Nucleus pulposus tissue was removed from coccygeal intervertebral discs and cells released by enzymatic digestion with 0.05% trypsin EDTA. They were cultured monolayer without serum in 6 well culture discs and cells released by enzymatic digestion with 0.05% trypsin EDTA. They were cultured monolayer without serum in 6 well culture discs and cells released by enzymatic digestion with 0.05% trypsin. Nucleus pulposus tissue was removed from coccygeal intervertebral discs and cells released by enzymatic digestion with 0.05% trypsin. Nucleus pulposus tissue was removed from coccygeal intervertebral discs and cells released by enzymatic digestion with 0.05% trypsin EDTA. They were cultured monolayer without serum in 6 well culture discs and cells released by enzymatic digestion with 0.05% trypsin.

Results
Figure-1

Figure-2

Figure-3

Discussion
Accerelation of spontaneous resorption of herniated intervertebral disc may have a great clinical relevance since patient undergo operation for not being endure time concerning conservative treatment. MMP-3 and MMP-7 has been reported to play a major role in TNF-alpha and MCP-1 pathways in resorption process of herniated intervertebral disc [2]. Iwabuchi et al has shown that LIPUS stimulation upregulate secretion of TIMP-1 in nucleus pulposus and enhance the maturation of MMP-3 [1]. Result of the current study showed that LIPUS stimulation to the nucleus pulposus cells enhance TIMP-1, antagonist of MMP-3, which is reported as another important mediator in the resorption process [3]. In LIPUS stimulation of macrophages, production of MCP-1 was enhanced which proved that LIPUS stimulation alone could induce macrophage activation and increase MCP-1 production. These data support the positive effect of LIPUS stimulation in acceleration of biological activity in nucleus pulposus and macrophage during resorption process. From RT-PCR results, we found that optimal duration of LIPUS stimulation in activating these two cells individually may be at 2-3 hours respectively.

Conclusion
LIPUS stimulation significantly activated nucleus pulposus cells and macrophage towards enhancement of TIMP-1 and MCP-1 at protein and gene level. LIPUS stimulation is a non-invasive and economical treatment that may be useful in accelerating spontaneous resorption of herniated intervertebral disc, which may be of great clinical benefit.

References