EFFECTS OF LOW INTENSITY ULTRASOUND ON THE CHONDROGENIC DIFFERENTIATION AND VIABILITY OF HUMAN MSCs FROM BONE MARROW IN ALGINATE CULTURE

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INTRODUCTION
Mesenchymal stem cells (MSCs) are undifferentiated pluripotential cells capable of differentiating into many cell types, such as osteoblasts, chondrocytes, adipocytes, and myocytes. MSCs are emerging as a promising intervention to treat various cartilage diseases and autologous cell source for cartilage tissue engineering. To date three-dimensional (3-D) culture and transforming growth factor-β (TGF-β) are known as the most essential factors among various factors for chondrogenic differentiation such as growth factors, cytokines and mechanical stimulation. However, the 3-D culture does not support even supply of nutrients and TGF-β treatment also reduces cell viability, particularly when using autologous MSCs from aged and/or osteoarthritis (OA) patients.

Low-intensity ultrasound (LIUS) was shown to enhance the repair of articular cartilage and fracture healing in vitro and in animal models. Recently, we have suggested that LIUS enhanced the chondrogenic differentiation of rabbit MSCs by itself even without TGF-β treatment (Cui et al., 2006). Here, we hypothesized that the LIUS treatment could enhance not only the chondrogenic differentiation but also the viability of human MSCs in vitro. Human MSCs from the bone marrow of OA patients were induced into the chondrogenic differentiation with or without TGF-β treatment in a 3-D alginate culture system, while the LIUS stimulation was applied with or without TGF-β treatment. The effects of LIUS on the expression of chondrogenic phenotypes and the cell viability in relation to apoptosis was addressed during the chondrogenic differentiation of hMSC.

MATERIALS AND METHODS
Human MSCs (hMSCs) were isolated from the bone marrow and cultured first in monolayer in α-MEM supplemented with 10% FBS. hMSCs were then induced to chondrogenic differentiation on a 3-D alginate in a serum free defined medium without TGF-β1. LIUS stimulation was performed for 20 minutes everyday for 7 consecutive days at 1 MHz and 200 mW/cm² in a continuous wave fashion with or without TGF-β1. The chondrogenic differentiation of hMSCs was examined by RT-PCR analysis for collagen type II expression. The viability of hMSCs was assessed by trypan blue exclusion and a Live/Dead assay kit (Molecular probes). Apoptosis of hMSCs was examined by a TUNEL assay kit (Calbiochem) and the expression of apoptosis-related proteins such as p53, bax, and bcl-2.

RESULTS
Effects of LIUS on the chondrogenesis of hMSCs: Type II collagen is a specific component of hyaline cartilage, while type I collagen is a typical marker for non-hyaline cartilage and is classically taken as a marker for dedifferentiated chondrocytes in culture. The collagen type II expression was increased at the mRNA level by LIUS stimulation, and it was paralleled with a decrease in the expression of collagen type I. In addition, the expression of another chondrogenic markers such as Sox-9 and aggrecan was also by induced by LIUS stimulation. Interestingly, LIUS stimulation alone increased their expression without TGF-β1 treatment.

Effects of LIUS/TGF-β treatment on hMSCs viability: In alginate culture, the viability of TGF-β treated hMSCs was significantly decreased (37% after 1 week, 28% after 2 weeks). However, it was increased by the LIUS stimulation (48% after 1 week, 42% after 2 weeks), when assessed by trypan blue exclusion or staining with the Live/Dead assay kit. The green color indicates the viable cells and the red color is dead cells. (Fig 1.)

Fig 1. Effects of LIUS on the hMSCs viability by Live/Dead Viability/ Cytotoxicity assay. (a) The green color indicates viable cells and red color indicates dead cells in the stained images. (b) The percentage of viable cells was presented from five independent experiments (n=5).

Fig 2. Effects of LIUS on the expression of apoptosis and cell viability related genes. (a) mRNA level of bax (181 bp) and bcl-2 (248 bp) was measured by RT-PCR. (b) Protein levels of p53 (53kDa), bax (21kDa), bcl-2 (24-26 kDa) and PCNA (36kDa) were measured by Western blot analysis.

CONCLUSION
These findings demonstrate that LIUS enhanced the chondrogenic differentiation and viability of human bone marrow MSCs in the alginate culture system. In particular, LIUS could induce at significant levels the chondrogenesis of hMSCs even without TGF-β treatment in our 3-D alginate culture system in vitro. The increase in the viability of hMSCs appeared to be mediated at least in part via the inhibition of cell apoptosis by LIUS as evidenced by the TUNEL assay and the decrease in the bax/bcl-2 ratio.

REFERENCES

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