EFFECTS OF DYNAMIC COMPRESSIVE LOADING ON CHONDROGENESIS OF RABBIT BONE MARROW-DERIVED MESENCHYMAL STEM CELLS

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INTRODUCTION

When injuries on arrticular cartilage extended into the subchondral bone, cells from the bone marrow (BM) migrate up to the injury area and form new cartilage-like tissue [2]. It indicates that chondrogenic differentiation of bone marrow cells can be induced by the biomechanical and biochemical stimuli at the injured site.

A recent cartilage repair study of rabbit keen joints showed that six months after the transplantation of BM-derived MSCs (BM-MSCs), different local mechanical environments resulted in substantial differences in mechanical properties of reparative tissues on the posterior and anterior aspects of the repair area [6]. It suggests that chondrogenic differentiation of BM-MSCs could be influenced by mechanical stimuli. The previous *in-vitro* studies have demonstrated that the treatment of transforming growth factor-β (TGF-β) can induce chondrogenesis of BM-MSCs [4,7]. Furthermore, mechanical loading studies of agarose cultures demonstrated that compressive loading stimulated chondrogenic differentiation of chick and mouse embryonic MSCs [3,5] as well as modulated the cartilage-specific macromolecule biosynthesis of mature chondrocytes [1]. However, the effects of physical stimulus associated with the mechanical environment of articular cartilage on chondrogenesis of BM-MSCs still remain unclear. The hypothesis of this study is that cyclic compressive loading can promote chondrogenesis of BM-MSCs in the absence of cytokines. Therefore, the objective of this study is to examine the effects of cyclic compressive loading on chondrogenic differentiation of rabbit BM-MSCs in agarose cultures.

MATERIALS AND METHODS

Isolation of Rabbit BM-MSCs Rabbit BM was harvested from the tibias of three New Zealand White rabbits (3 months old) and aspirated into a 10-ml syringe containing 1 ml of heparin (3000 U/ml). The aspirate was transferred into 50-ml tube and centrifuged at 600xg for 10 min. The cells were resuspended in 10 ml of low-glucose Dulbecco's modified eagle's medium (DMEM) (GibcoBRL, Grand Island, NY). A small aliquot of this cell suspension was mixed with an equal volume of 4% acetic acid to lyse the red blood cells and the number of nucleated cells was counted with a hemocytometer. The cells were plated in 10-cm dish at a density of 1x 10^5 cells and cultured with low-glucose DMEM containing 10% fetal bovine serum (FBS) and 1% antibiotics at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. After 5 days of culture, nonadherent cells were removed by changing culture medium. The BM-MSCs were expanded by re-plating into three 10-cm culture disks after 20 days of culture.

Preparation of Cell-Agarose Constructs After trypsinizing and cell counting, rabbit BM-MSCs were suspended in high-glucose DMEM, and then mixed with an equal volume of 4% (wt/vol) agarose solution at 37°C to produce mixtures of $5x10^6$ cells/ml. The cell-agarose constructs (8 mm in diameter and 1.5 mm thick) were formed by casting cell-agarose mixture in a custom-designed mold and gelling for 15 min at room temperature.

Chondrogenic potential of rabbit BM-MSCs was initially examined by agarose cultures. The cell-agarose constructs of the control group (n=6) were cultured in defined serum-free medium consisting of high-glucose DMEM, 1% Insulin-Transferrin-Selenium supplements (GibcoBRL; final concentrations: 10 μg/ml bovine insulin, 5.5 μg/ml transferrin, 6.7 ng/ml sodium selenite), 1.25 mg/ml bovine albumin, 5.33 μg/ml linoleic acid, 40 μg/ml proline, 50 μg /ml ascorbic acid, and 10^{-7} M dexamethasone (Sigma, St. Louis, MO), while the serum-free medium supplemented with 10 ng/ml TGF-β1 (R&D Systems, Minneapolis, MN) was used for the treated group (n=6). All agarose cultures were performed in a humidified incubator maintained at 37°C in 5% CO_2. The expressions of cartilage-specific markers (collagen type II and aggrecan) and collagen type I gene were examined on cell-agarose constructs after a 21-day culture. The culture medium was changed every 2-3 days.

Bioreactor system A bioreactor was developed to induce mechanical stimuli within agarose specimens under unconfined dynamic compressive loading. In the bioreactor, twelve specimens can be subjected to dynamic compressive loading simultaneously between porous filters and an impermeable platen while the load response of specimens and the imposed displacement can be measured by a load cell and a LVDT, respectively (Figure 1). In order to

maintain the viability of cells, the testing chamber was filled with culture medium during testing while the bioreactor performed the experiments in a humidified incubator maintained at 37°C in 5% CO_2.

Dynamic Unconfined Compression Tests After cell-agarose construct preparation, specimens were cultured in a serum-free medium, UltraCULTURE (Biowhittaker, Walkersville, MD), containing 1% antibiotics overnight (20-24 h) and then divided into three groups: control (n=9), TGF-β1 treated (n=9), and dynamic loading (n=9) groups. Specimens of the dynamic loading group were placed into the testing chamber of the bioreactor filled with UltraCULTURE. After tare loading of 5% strain, sinusoidal compressive loading was applied to the specimens with the magnitude of 10 % strain at the frequency of 1 Hz. The loading experiment was carried out 4 hr/day for 3 days. During the testing period, the specimens of the control and TGF-β1treated groups were cultured in UltraCULTURE without and with the supplement of 10 ng/ml TGF-β1, respectively. The expressions of cartilage-specific markers and collagen type I gene were analyzed for three groups after a 3-day testing.

Reverse Transcription–Polymerase Chain Reaction (RT–PCR) Analysis The gene expression of Rabbit BM-MSCs was examined by the RT-PCR analysis. The total RNA was extracted from cell-agarose constructs using the reagent Trizol (GibcoBRL) according to the manufacturer's instructions. The RT-PCR was performed in GeneAmp PCR system (Perkin Elmer Ceyus, Norwalk, CT) using the ThermoScript RT-PCT system (GibcoBRL). The PCR primers are shown in Table 1. As an internal control, the constitutively expressed housekeeping gene, β-actin, was also synthesized. The PCR products were analyzed by electrophoresis on 2% agarose gel containing ethidium bromide and photographed using a low light image system (ChemiImager 4000, Alpha Innotech Corporation, San Leandro, CA).

Gene	Size/Sequence
Collagen I	827 bp
(Sense)	5'-CGTGGTGACAAGGGTGAGAC-3'
(Antisense)	5'-TAGGTGATGTTCTGGGAGGC-3'
Collagen II	366 bp
(Sense)	5'-GCACCCATGGACATTGGAGGG-3'
(Antisense)	5'-GACACGGAGTAGCACCATCG-3'
Aggrecan	313 bp
(Sense)	5'-GAGGAGATGGAGGGTGAGGTCTTT-3'
(Antisense)	5'-CTTCGCCTGTGTAGCAGCTG-3'
β-actin	353 bp
(Sense)	5'-GCTCGTCGTCGACAACGGCTC-3'
(Antisense)	5'-CAAACATGATCTGGGTCATCTTCTC-3'

Table 1 Sequences of PCR primers

RESULTS AND DISCUSSION

After a 21-day agarose culture, the TGF-β1 treated specimens exhibited stronger expressions of chondrogenic markers (collagen type II and aggrecan) and weaker expression of collagen type I gene than the specimens without the treatment of TGF-β1 (Figure 2a). It demonstrates that the agarose culture is able to support chondrogenesis of rabbit BM-MSCs under the treatment of TGF-β1.

In the compression study, the specimens of the dynamic loading group exhibited stronger expression of collagen type II gene than the specimens of the TGF-β1 treated and control groups while all specimens expressed collagen type I gene. However, the expression of aggrecan gene was not detected in specimens of the three groups after 3 days of culture.

Stronger expression of collagen type II gene exhibited by specimens under dynamic loading suggests that dynamic compressive loading may promote chondrogenesis of rabbit BM-MSCs. The future studies will examine the short- and long-term effects of dynamic loading with different loading parameters (magnitude, frequency, and duration) on chondrogenesis of BM-MSCs.

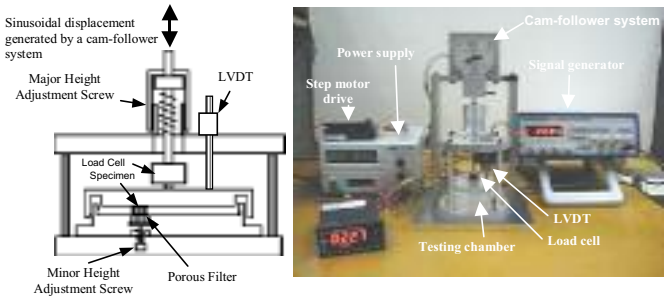

Figure 1 Schematics of the bioreactor.

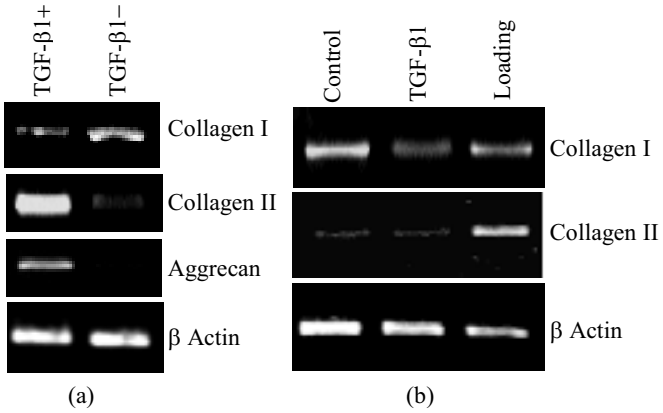

Figure 2 Typical gene expressions of rabbit MB-MSCs (a) after a 21-day agarose culture with or without the treatment of TGF-β1 and (b) after a 3-day agarose culture with or without the treatment of TGF-β1 or with being subjected to dynamic compressive loading.

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