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An Electromagnetic Compressive Force by Cell Exciter Stimulates Chondrogenic Differentiation of Bone Marrow–Derived Mesenchymal Stem Cells

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ABSTRACT

In this study, we present a biological micro-electromechanical system and its application to the chondrogenic differentiation of rabbit bone marrow–derived mesenchymal stem cells (MSCs). Actuated by an electromagnetic force, the micro cell exciter was designed to deliver a cyclic compressive load (CCL) with various magnitudes. Two major parts in the system are an actuator and a cartridge-type chamber. The former has a permanent magnet and coil, and the latter is equipped with 7 sample dishes and 7 metal caps. Mixed with a 2.4% alginate solution, the alginate/MSC layers were positioned in the sample dishes; the caps contained chondrogenic defined medium without transforming growth factor-β (TGF-β). Once powered, the actuator coil-derived electromagnetic force pulled the metal caps down, compressing the samples. The cyclic load was given at 1-Hz frequency for 10 min twice a day. Samples in the dishes without a cap served as a control. The samples were analyzed at 3, 5, and 7 days after stimulation for cell viability, biochemical assays, histologic features, immunohistochemistry, and gene expression of the chondrogenic markers. Applied to the alginate/MSC layer, the CCL system enhanced the synthesis of cartilage-specific matrix proteins and the chondrogenic markers, such as aggrecan, type II collagen, and Sox9. We found that the micromechanically exerted CCL by the cell exciter was very effective in enhancing the chondrogenic differentiation of MSCs, even without using exogenous TGF-β.

INTRODUCTION

MESENCHYMAL STEM CELLS (MSCs) are undifferentiated pluripotent cells capable of differentiating into many cell types, such as osteoblasts, chondrocytes, adipocytes, myocytes, and neurons under specific defined culture conditions.[1,2] The MSCs hold great promise as an alternative to chondrocytes in cartilage tissue engineering because chondrocytes have a limitation in cell source and easily lose their phenotypes during expansion *in vitro*. However, efficient and controllable methods to induce chondrogenic differentiation should first be developed to use MSCs for cartilage tissue repair.

Identification of the appropriate biological signals is of particular interest in basic research to understand the mechanism in the regulation of MSC proliferation and differentiation. Soluble growth factors, such as transforming growth factor-β (TGF-β), bone morphogenic protein-2 (BMP-2),

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and fibroblast growth factor-2 (FGF-2), have been examined for their roles in differentiating MSCs into chondrogenic cells.[3–5] Meanwhile, biomechanical cues have also been widely investigated using various 3-dimensional (3D) models of chondrocyte culture.[6–8] The stimulatory effects have been documented in numerous studies that found the cartilage-specific extracellular matrix molecules upregulated under appropriate mechanical loading conditions.[9,10] When the same principle was extended to MSCs, these molecules were proved to be effective in the induction of chondrogenic differentiation of MSCs as well. For example, cyclic hydrostatic pressure could enhance the biosynthesis of ECM during the chondrogenesis of rabbit[11] and human MSCs.[12–14] Dynamic compressive load also promoted chondrogenesis of embryonic limb-bud MSCs.[15] Recently, we reported that low-intensity ultrasound, a possible source of mechanical stimulation, enhanced chondrogenic differentiation of MSCs *in vivo* and *in vitro*.[16,17]

In the present work, we introduce a novel biological microelectromechanical system (BioMEMS) that is intended to produce a cyclic compressive load (CCL) and thus to mimic the stimulatory effects on chondrogenesis shown in large-scale mechanical devices. The present instrument is designed to use an electromagnetic force to generate a dynamic compression load. The primary benefit of the system is the application of micromechanical stimulation (approximately 10 KPa),[18,19] as opposed to conventional systems, which use a much greater magnitude of hydrostatic and compression load.[14,15] Another benefit is that multiple samples (up to 7) can be tested simultaneously under a controllable and uniform condition for such variables as load, frequency, and duration. We postulated that the device could be useful for the chondrogenic differentiation of MSCs and for investigating the role of mechanical signals in that process. To validate the current system, rabbit MSCs were prepared in the alginate layer and the effect of the CCL was examined on their chondrogenic differentiation with use of the device. Alginate was chosen as a cell matrix because it ensures that cells are uniformly distributed in the complex and can be easily retrieved after stimulation, enabling multiple sampling for quantitative and qualitative studies.

MATERIALS AND METHODS

Fabrication of electromagnetic cell exciter

As shown in Fig. 1A, the electromagnetic cell exciter consists of an actuator and a cartridge-type chamber. There are 7 magnetic actuators in the system, each of which is composed of a permanent magnet, core, and coil. The magnet is a cylinder form with an outer diameter of 9 mm and an inner diameter of 6 mm. The core is wound with 350 turns of enameled copper wire (diameter, 100 μm). Fabricated with poly-methyl-methacrylate, the chamber contains 7 sample wells for cell culture, each 12 mm in diameter and 5 mm in height. Once the system is assembled (Fig. 1B), individual samples are placed in each well and covered with a metal cap (1 g) on the top. The metal caps are 1.5 mm thick and composed of 2 disks; the upper disk (diameter, 3 mm) is permanently magnetized with neodymium iron boron (2900G) and the lower disk (diameter, 12 mm) is treated only with epoxy coating to prevent rust without magnetization.

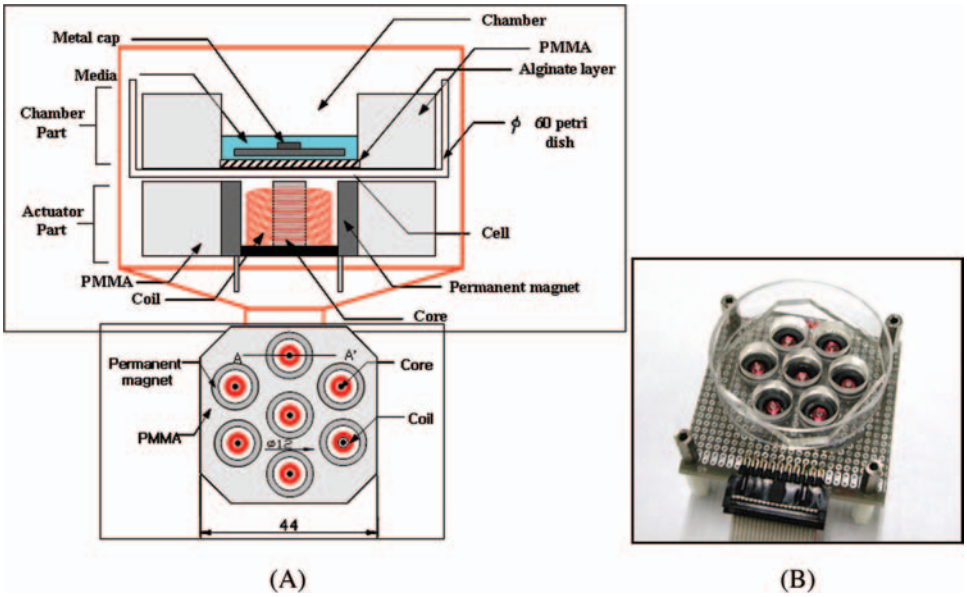

FIG. 1. Illustration of the electromagnetic compressive cell exciter (**A**) and its digital image (**B**). The exciter is composed of an actuator and a cartridge-type chamber. Each of the 7 magnetic actuators in the system contains a permanent magnet, core, and coil. Fabricated with poly-methyl-methacrylate (PMMA), the chamber contains 7 dishes, each 12 mm in diameter and 5 mm in height. Color images available online at www.liebertpub.com/ten.

Isolation of bone marrow–derived rabbit MSCs

The bone marrow aspirates were obtained aseptically from the tibia and femur of 2-week-old New Zealand white rabbits (Joong-Ang Experimental Animal Center, Seoul, Korea), as described elsewhere.[16,20] By using a cell strainer (70 μm nylon, Falcon, Franklin Lake, NJ), the bone marrow aspirates were filtered, pooled, and centrifuged at 500 *g* for 5 min to remove the supernatants. The cell pellet was resuspended in 20 mL of α-minimum essential medium (α-MEM) (Sigma, St. Louis, MO). The cells in α-MEM were supplemented with 1% antibiotic-antimycotic (GIBCO BRL, Grand Island, NY) and 10% newborn calf serum (GIBCO BRL). For cell counting, a small aliquot of cells was suspended in 5% acetic acid to remove red blood cells, and mononuclear cells were counted by trypan blue exclusion. After counting, 1.5×10^7 cells were seeded in a culture dish (150 mm) and cultured as a monolayer for 10–14 days in a humidified incubator at 37°C under 5% carbon dioxide. When confluent, cells were detached by trypsin treatment and cultured again as described previously before they were used for the 3D alginate layer culture.

Preparation of alginate/MSC layer

The MSCs were suspended in a sodium alginate (Sigma) solution (2.4% in 0.15 M sodium chloride [NaCl]) at a density of 5×10^6 cells/mL. Before mixing with cells, the alginate solution was autoclaved at 120°for 30 min for sterilization. The alginate/MSCs mixture (300 μL of each) was transferred onto the semipermeable membrane in the transwell insert (Millipore Corp., Billerica, MA) and spread evenly over the surface. The transwell insert was allowed to immerse in 1 mL of sterile 100 mM calcium chloride solution for 10 min to produce a semisolid alginate/MSC layer 3 mm thick. Each layer was washed twice with 0.15 M NaCl and finally rinsed in chondrogenic defined medium (CDM), which contained 1% antibiotic-antimycotic, 6.25 μg/mL insulin, 6.25 μg/mL selenious acid, 6.25 μg/mL transferrin, 50 μg/mL ascorbic acid, 100 nM dexamethasone, 40 μg/mL proline, 1.25 mg/mL bovine serum albumin (BSA), and 100 μg/mL sodium pyruvate. Each sample was then located in the chamber well for the test. The materials for CDM were purchased from Sigma. The CDM in this experiment did not contain TGF-β.

Application of CCL

Once the setup was complete, an input (1A and 5V) was applied to the actuator coil so that electromagnet-derived force could be generated.[19] It attracted the metal caps and produced a compressive stress on the samples. To assess the effect of mechanical stimulation on the chondrogenesis of rabbit MSCs, the alginate/MSC layer was exposed to the cyclic compressive load at 1-Hz frequency for 10 min twice a day for 7 days (CCL group). Samples in the control group remained uncapped in the chamber well and thus received no mechanical load, although the electromagnetic field was on during the experiment. Samples were then cultured at 37°C in a humidified incubator with a 5% carbon dioxide supply. The culture medium was changed daily.

Measurement of cell viability

For the assay of cell viability in the alginate/MSC layer, a Live/Dead/Viability/Cytotoxicity kit (Molecular Probes, Eugene, OR) was used. Following the manufacturer's instruction, the alginate/MSC layers were rinsed with phosphate-buffered saline (PBS) and treated in a solution containing 2 mM ethidium homodimer-1, PBS, and 4 mM calcein AM for 40 min. After being washed in sterilized PBS, a thin slice of the alginate layer was placed on the slide and then observed by fluorescent microscopy. As live cells appeared in green and dead cells in red, the viability of cells was measured by dividing the number of viable cells (green cells) with that of total cells (green cells + red cells).

Biochemical assays

The recovered samples ($n=4$ for each group) were digested for 16 h in a papain digestion solution (125 μg/mL papain, 5 mM L-cystein, 100 mM sodium phosphate, 5 mM ethylenediaminetetraacetic acid, pH 6.2) at 60°C. Total glycosaminoglycan (GAG) contents were analyzed using the 1, 9-dimethylmethylene blue (DMMB) assay.[21] Individual samples were mixed with the DMMB solution, and the absorbance was measured using ELISA READER (BioTek, Inc., Winooski, VT) at 525 nm wavelength. The total GAG of each sample was extrapolated by using a standard plot of shark chondroitin sulfate (Sigma) in the range of 0–5 μg/mL. The collagen content was measured using a modified Hride Tullberg-Reinert method.[22] The digested samples ($n=4$ for each group) were dried at 37°C in the 96-well plate for 24 h and then reacted with a dye solution for 1 h on a shaker. The dye solution (pH, 3.5) was prepared with Sirius red dissolved in picric acid–saturated solution (1.3%; Sigma) to a final concentration of 1 mg/mL. After being washed 5 times with 0.01 N hydrogen chloride, the dye-sample complex was dissolved in 0.1 N sodium hydroxide. The absorbance was read at 550 nm wavelength. The total collagen of each sample was extrapolated by using a standard plot of bovine collagen (Sigma) in the range of 0–10 μg/mL. To avoid variations from scaffold sizes, the GAG and collagen contents were normalized against total cell numbers, represented by the total DNA content measured by the Hoechst 33258 DNA assay (Strasbourg, France).[23]

The papain digested samples (20 μL each) were transferred into 96-well plates in duplicates and mixed with 200 μL of 40 ng/mL Hoechst 33258 dye. Samples were read with an LS55 luminescence spectrometer (PerkinElmer, Boston, MA) at 346 nm excitation and 460 nm emission. A standard curve was generated using 5, 10, 20, 40, 60, 80, and 100 ng of double-stranded calf thymus DNA.

Histologic and immunohistochemical evaluation

The alginate/MSCs layers were rinsed with PBS and polymerized in barium chloride, followed by 10% formalin fixation for 24 h. The samples were then embedded in paraffin and divided into 4-μm-thick sections. Serial sections were stained with Safranin-O and Alcian blue for sulfated proteoglycan in the matrix. Immunohistochemistry was also carried out to measure the expression of type II collagen, a typical extracellular matrix protein of the hyaline cartilage. After being washed sequentially in 70% ethanol and PBS, the sections were treated briefly with 3% hydrogen peroxide in PBS and then with 0.15% Triton X-100. Once blocked with 1% BSA solution, they were reacted with monoclonal antibody of mouse anti-human type II collagen (1:500; Chemicon, Temecula, CA) for 1 h, followed by the addition of the biotinylated secondary antibody (1:200; Chemicon). The signals were detected using the Universal DakoCytomation Labelled Streptavidin-Biotin2 System (Dakocytomation, Glostrup, Denmark). The detection system is based on the modified labeled avidin-biotin technique, coupled with horseradish peroxidase and 3-3′ diaminobenzidine substrate-chromogen, which results in a brown precipitate. The immunostained sections were counterstained with Mayer's hematoxylin (Sigma) before microscopic examination (Nikon E600, Nikon, Tokyo, Japan). The images from the control and loaded samples were analyzed using Image-ProPlus version 5.0 (Media Cybernetics, Silver Spring, MD) to count the number of type II collagen–positive spots for quantitative analysis. Five different fields were randomly selected.

Reverse transcriptase polymerase chain reaction analysis

By using Trizol reagent (Invitrogen, Carlsbad, CA), total RNA was extracted from cells recovered from the alginate layer. The RNA pellet was briefly dried and then dissolved in diethylpyrocarbonate water. A total of 1 μg of RNA was used for the first strand complementary DNA (cDNA) synthesis by using Superscript First Strand Synthesis System (GIBCO), and 2 μL of the synthesized cDNA was used for polymerase chain reaction (PCR) by using primers listed in Table 1. All PCR reactions were performed for 35 cycles, and the annealing temperatures for primer sets are described in Table 1. Type II collagen, Sox9, and aggrecan were used for chondrogenic markers, and type I collagen, alkaline phosphatase, and osteopontin were used for osteogenic markers. Glyceraldehyde-3-phosphate dehydrogenase was used as an internal control. The PCR products were separated on a 1.5% agarose gel, visualized by ethidium bromide staining, and then photographed using a low-light image system (GelDoc 2000, BioRad, Hercules, CA).

Statistical analysis

Each set of experiments ($n = 4$ for each group) was performed at least 5 times. Data are presented as the mean ± standard deviation from the result of a representative experiment. Comparison between the control and experimental groups was evaluated by one-way analysis of variance (ANOVA) using GraphPad Instat software (GraphPad Software Inc., San Diego, CA).

RESULTS

Cell viability

The MSCs/alginate layer cultures were untreated or treated with the CCL at 1-Hz frequency for 10 min twice a day. The control and CCL groups were stained at 0, 3, 5, and 7 days after stimulation for cell viability using the Live/

Table 1. PCR Primer Sequences for Specific Genes

Gene	*Primer*	*Sequence*	*Annealing temp. (°C)*
PCR primer sequences for chodrogenesis markers.			
GAPDH	Sense	5′-caacttttcagagggacaag-3	55
	Antisense	5′-ctaaaccatccaatcggtag-3′	
Aggrecan	Sense	5′-gaaaggtgttgtgttccact-3′	57
	Antisense	5′-gtcataggtctcgttggtgt-3′	
Type I collagen	Sense	5′-cttctggccctgctggaaaggatg-3′	55
	Antisense	5′-cccggatacaggtttcgccagtag-3′	
Type II collagen	Sense	5′-gatattgcacctttggacat-3′	55
	Antisense	5′-cccacaatttaagcaagaag-3′	
SOX-9	Sense	5′-cacacagctcactcgaccttg-3′	57
	Antisense	5′-ttcggttatttttaggatcatctcg-3′	
PCR primer sequences for osteogenesis markers.			
ALP	Sense	5′-atgatttcaccattcttagtactg-3′	55
	Antisense	5′-tcagaacaggacgctcagggg-3′	
Osteopontin	Sense	5′-agctttacaacaaatacccagatg-3′	55
	Antisense	5′-taggagattctgcttctgagatg-3′	

ALP: alkaline phosphatase, GAPDH: glyceraldehyde-3-phosphate dehydrogenase, PCR: polymerase chain reaction.

Dead/Viability/Cytotoxicity assay. The live and dead cells were stained in green and red, respectively. As measured by the ratio of the number of green cells to that of the total cells (green cells + red cells), the cell viability of the samples was slowly decreasing to about 80% on average over 1 week in both groups (Fig. 2). Therefore, there was no statistical difference between groups when measured from 5 independent experiments. This indicated that the present CCL stimulation had no influence on the viability of MSCs in alginate layer culture.

Histologic and immunohistochemical analysis

To identify the formation and distribution of the cartilage-specific ECM molecules, the thin sections of each specimen were subjected to both Safranin-O and Alcian blue stainings. While the cells seemed homogeneously distributed within the matrix for the control and CCL stimulated samples, some notable differences were also apparent. From the Safranin-O staining, the chondrocyte-specific lacunae formation was clear and more widely distributed in the CCL group than in the control group (Fig. 3A). The Alcian blue staining revealed a similar effect of CCL, as presented by the higher concentration of sulfated proteoglycan accumulation around the cells in the CCL group, particularly at days 3 and 5 (Fig. 3B and C). At day 7, the proteoglycan accumulation was rapidly increasing in the control group but was unchanged in the CCL group; thus, the result was not statistically different between groups. Immunostaining results also showed that type II collagen was more actively produced in the CCL group at all time points of days 3, 5, and 7 (Fig. 4). When the type II collagen–positive spots were counted and normalized with the total cell numbers, there were statistically significant differences between the control and CCL groups at all time points (Fig. 4B). The highest value was found in the CCL group at day 3.

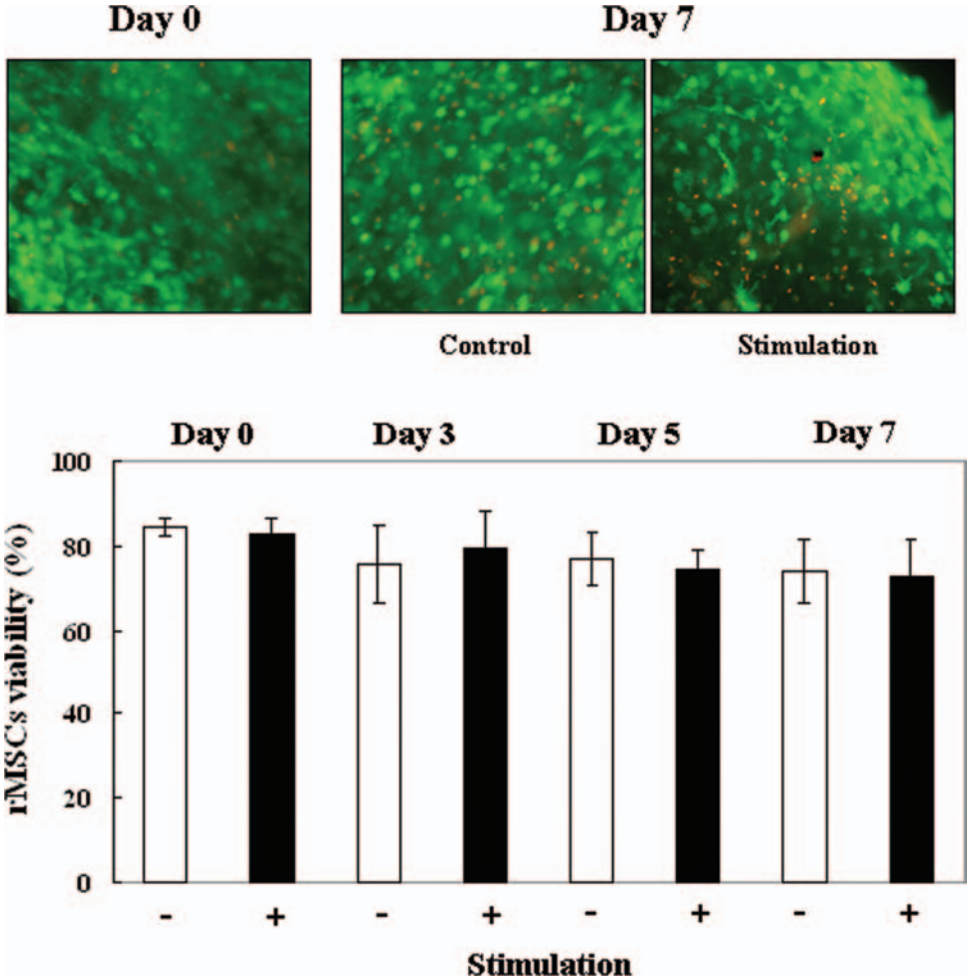

FIG. 2. Measurement of the viability of rabbit mesenchymal stem cells (rMSCs). (**Top**) Live/Dead/Viability/Cytotoxicity assay. Within the alginate layer, the living cells were stained green and the dead ones, red. Stained sections at days 0 and 7 are shown (original magnification, ×200). (**Bottom**) Measurement of cell viability. The viability of cells was measured by dividing the number of viable cells (green cells) with that of total cells (green cells + red cells). The viabilities were not statistically different between groups ($n = 4$ for each group). Color images available online at www.liebertpub.com/ten.

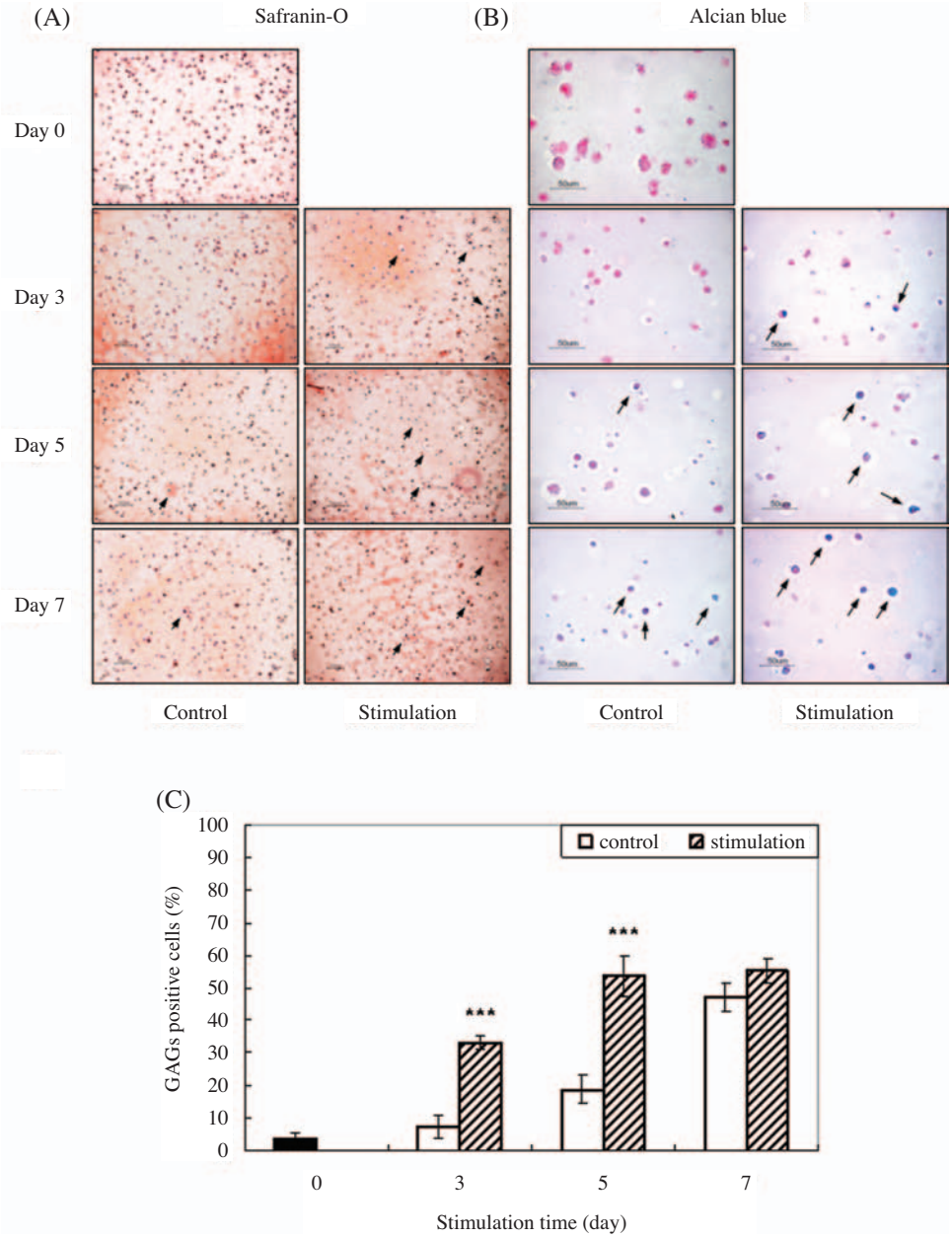

FIG. 3. Histochemical staining for sulfated glycosaminoglycan (GAG). Thin slices of specimens were subjected to the (**A**) Safranin-O staining (original magnification, ×100) and (**B**) Alcian blue staining (original magnification, ×400). As indicated by arrows, GAG was positively stained in red (Safranin-O) and blue (Alcian blue), respectively. (**C**) Percentage of cells positively stained on Alcian blue staining. ***$P < .001$ at each time point ($n = 4$). Color images available online at www.liebertpub.com/ten.

Reverse transcriptase PCR analysis

To further support the effect of CCL stimulation on chondrogenic induction of MSCs, the expression of genes involved in the chondrogenic and osteogenic differentiation was examined by reverse transcriptase PCR (RT-PCR). Sox9 is a key transcription factor in the chondrogenic differentiation. Its messenger RNA was detected clearly in response to CCL at day 3 (Fig. 5). The gene expression of both aggrecan and type II collagen was also induced in response to CCL similarly with the Sox9 expression, reaching a peak level at day 3 and decreasing gradually with time. In contrast, their expression was not detected clearly at all time points in the control group. The gene expression of type I collagen was initially high in the control group and was decreased by the CCL stimulation. The gene expression of the osteogenic markers, osteopontin and alkaline phosphatase, was not detected in either group.

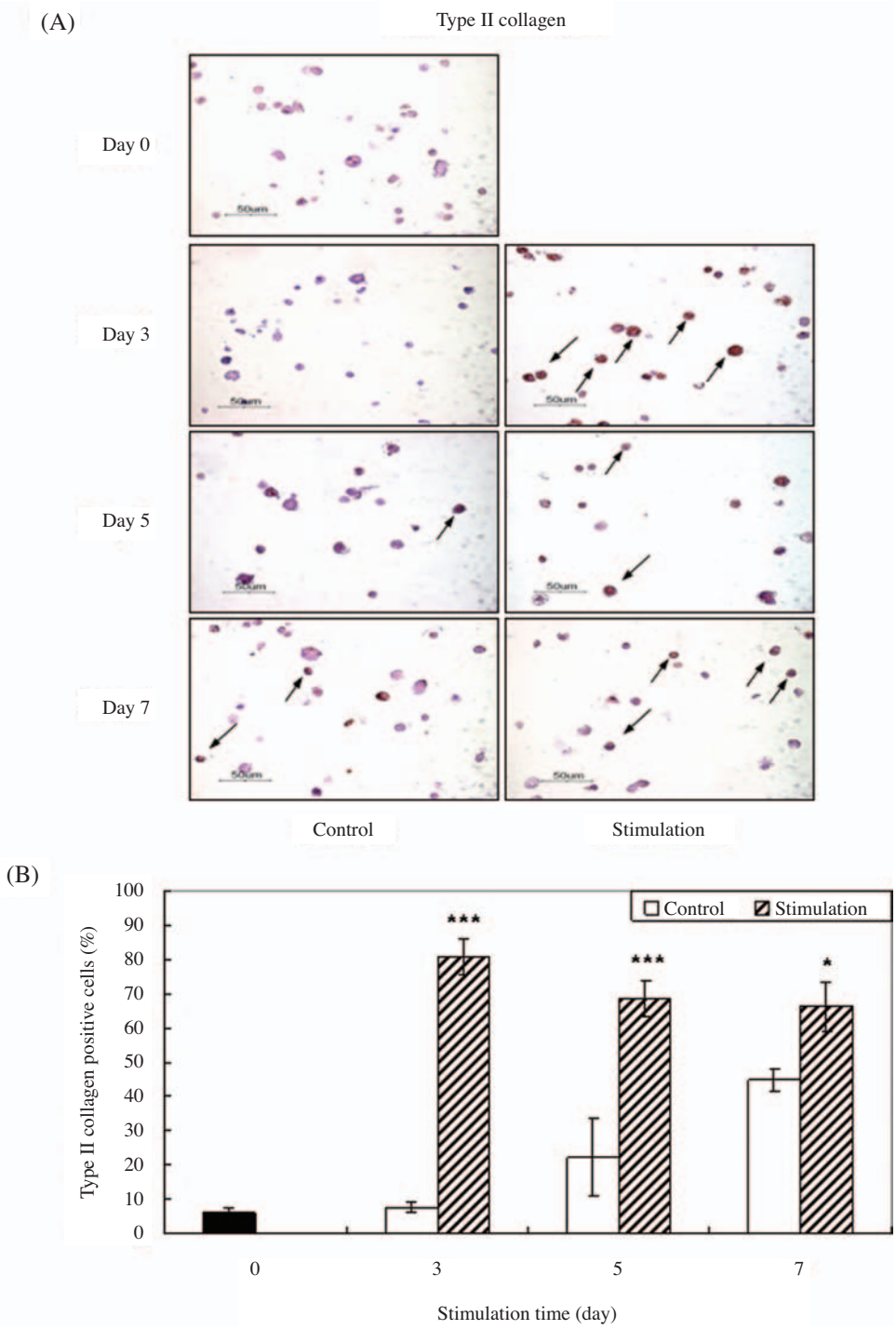

FIG. 4. Immunohistochemistry for type II collagen. (**A**) Cells positive for type II collagen (arrows) are brown (original magnification, ×400). (**B**) Percentage of cells positive for type II collagen. *$p < .05$ and ***$p < .001$ at each time point ($n = 4$). Color images available online at www.liebertpub.com/ten.

Total GAG and collagen contents

When the total amounts of GAG and collagen were measured at days 3, 5, and 7 after stimulation, these substances increased gradually with time only in the CCL group. Compared to the control, the differences were statistically significant at days 5 and 7 (Fig. 6). At day 7, for example, the average concentrations after normalization with total DNA contents (μg/μg DNA) in the control and CCL groups were 1.5 ± 0.12 μg and 2.9 ± 0.29 μg for GAG and 0.13 ± 0.02 μg and 0.23 ± 0.02 μg for collagen, re-

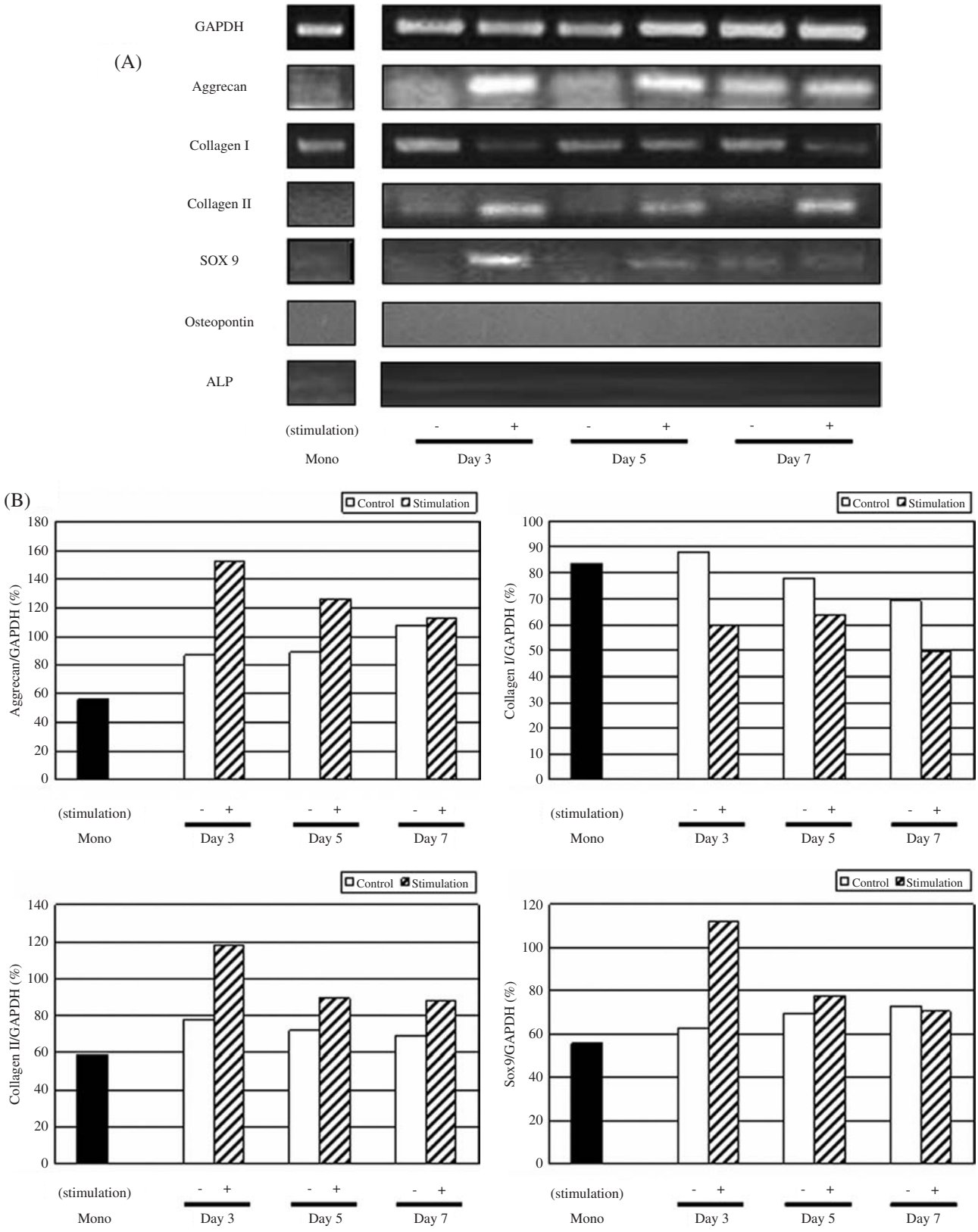

FIG. 5. Gene expression profiles for chondrogenic and osteogenic markers with or without the cyclic compression load. Target genes were aggrecan, type I, II collagens, Sox9, alkaline phosphatase, and osteopontin. Glyceraldehyde-3-phosphate dehydrogenase was used as a housekeeping gene. (**A**) Messenger RNA levels of the marker genes were measured by reverse transcriptase polymerase chain reaction analysis at days 3, 5, and 7. A representative image from several independent experiments is presented. (**B**) Histogram showing relative intensities of the signals. ALP: alkaline phosphatase, GADPH: glyceraldehyde-3-phosphate dehydrogenase.

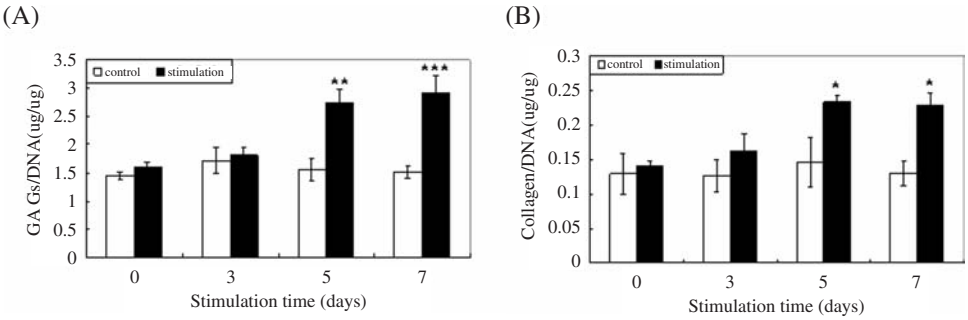

FIG. 6. Biochemical analysis of glycosaminoglycan (GAG) and collagen. Total contents of glycosaminoglycan (**A**) and collagen (**B**) were measured by 1, 9-dimethylmethylene blue assay and a modified Hride Tullberg-Reinert assay, respectively. The amounts were normalized by the DNA content of each specimen in the histograms. $*p < .05$, $**p < .01$, and $***p < .001$ at each time point ($n = 4$).

spectively. Average DNA contents at day 7 were not significantly different between groups; they ranged from $14.24 \pm 0.24\,\mu g$ to $14.93 \pm 0.34\,\mu g$ for GAG and from $14.51 \pm 0.39\,\mu g$ to $14.93 \pm 0.34\,\mu g$ for collagen. The patterns of results for GAG or collagen were therefore similar with or without normalization (data not shown).

DISCUSSION

Mechanical stimulations such as cyclic hydrostatic pressure have been reported to have a potential to modulate chondrogenesis of MSCs.[13,14] In this study, we demonstrated that a bioMEMS device that generates CCL could enhance the chondrogenic differentiation of rabbit MSCs. Applied to the alginate-MSCs layer, the CCL resulted in the synthesis of the cartilage-specific matrix molecules at higher levels (Fig. 3) and induced gene expressions of chondrogenic markers, such as aggrecan, type II collagen, and Sox9 (Fig. 5). In particular, the effects of CCL were shown even without TGF-β treatment, suggesting that CCL by itself could induce chondrogenic differentiation of MSCs. We speculate that CCL could be also helpful for primary chondrocytes in a 3D culture to retain their phenotypes.

To provide a 3D environment for the dynamic compression, an alginate layer was chosen as a support matrix because cells were uniformly distributed in the matrix and thus the homogeneity of individual samples could be ensured.[24,25] Although the alginate bead culture system has been more widely used for the chondrogenesis of MSCs, it was an inappropriate culture model for the CCL by bioMEMS in this study because of its weak mechanical property and uneven size.

Sox9 is a key transcription factor for chondrogenic differentiation and cartilage formation, and its expression is believed to precede the upregulation of cartilage-specific genes during *in vitro* chondrogenesis.[26,27] It is also widely accepted that Sox9 responds to mechanical cues to induce chondrogenesis. Takahashi *et al.*[15] demonstrated that the compressive force increased Sox9 in the chondrogenesis of mouse embryonic limb-bud MSCs. Huang *et al.*[11] showed that the compressive load could upregulate the chondrogenic markers, including Sox9, and contribute to the increase in endogenous TGF-β secretion. According to the RT-PCR results, the gene expression of Sox9 was notable only in the CCL group. The expression pattern of Sox9 by the CCL stimulation coincided well with those of aggrecan and type II collagen (Figs. 5 and 6). These results imply that the Sox9 expression was responsive to the mechanical signals from the CCL at levels sufficient to induce the expression of type II collagen and aggrecan. Because messenger RNA levels of osteogenic markers, osteopontin, and alkaline phosphatase were not detectable, the possibility of osteogenesis seems negligible in the present system.

Because TGF-β was not used in this study, our results are independent of this well-known inducer of chondrogenesis. Our findings therefore suggest that the CCL itself (in combination with the 3D environment) could be a chondrogenic signal for MSCs. Although many studies have documented that TGF-β is effective for chondrogenic differentiation,[28,29] others argued that TGF-β alone may not be sufficient.[30,31] They found that 10 ng/mL TGF-β was ineffective by itself and should be combined with either cartilage-derived morphogenetic protein-1 or BMP-2 to efficiently induce the chondrogenic differentiation of MSCs in pellet culture. However, Bosnakovski *et al.*[32] reported that chondrogenesis of bovine MSCs in pellet culture occurred without addition of any growth factors. The conflicting results on the effects of TGF-β seemed to be due mainly to different cell sources, cell origin, and culture environment. The use of TGF-β1 may be of interest in future studies of whether the present system has synergistic effects with TGF-β on the progression of chondrogenic differentiation of MSCs.

Since an electromagnetic field was created in the present setting, we speculate that the unidentified effects of electromagnetic field to MSCs may be reflected in the final outcomes. It does not seem, however, that the current electro-

magnetic field has any notable effects on the chondrogenesis of MSCs. We compared 2 control groups, one the electromagnetic field–exposed alginate/MSC sample without a metal cap and the other is the electromagnetic field–free alginate/MSCs with a metal cap. The comparative results for type II collagen expression were not significantly different between the control groups (data not shown). Therefore, the direct effect of electromagnetic field exposure appeared to be negligible, and it is conceivable that the CCL stimulation was the major contributor to the present results. Although direct measurement of the intensity of electromagnetic field was impossible, the test results of the controls were sufficient to suggest little effect of the present electromagnetic field on the differentiation of MSC as mentioned previously.

Although numerous studies have examined the effects of electromagnetic field on differentiation and gene expression of chondrocytes, few studies have assessed MSCs.[33,34] With the cartilage explants, an electromagnetic field (75 Hz, 1.5 mT) significantly increased not only the ^{35}S-sulfate incorporation and the cartilage-specific proteins levels but also messenger RNA of aggrecan and type II collagen. Compared to our electromagnetic field system, these studies used much higher frequencies and intensities. In our previous study, various factors (frequency, load, and time) were tested for the optimal CCL condition with the viability and type II collagen expression of MSCs.[19] Among multiple frequencies examined, ranging from 0.1 to 1000 Hz, 1 Hz was the most chondrogenic-effective frequency in terms of type II collagen production. Validated by using a laser displacement meter (LC-2420, Keyence, Milton Keynes, UK), the magnitude of the compressive load was calculated using deflection and Young's modulus of a standard material. The effective load was set to 10 kPa because alginate gel was cracked by CCL greater than 100 kPa. In addition, the stimulation condition for 10 min twice a day was more effective in the chondrogenesis of MSCs than was continuous stimulation.

The magnitude of the applied stress was estimated at 5–10 kPa, which was significantly lower than values in the conventional mechanical loading protocols. Considering the physiologic stress level (0.1–20 MPa) within the normal articular cartilage, the applied load in the present system is much more similar to the actual situation, even though the cell type was different (chondrocytes vs. MSCs). Few studies have examined the proper level of mechanical stress, including the magnitude, duration, and frequency that could mimic the natural condition of chondrogenic development of MSCs *in vivo*. More systemic tests are thus necessary to validate the current instrument.

In conclusion, the CCL generated from the electromagnetic force–derived cell exciter could be an effective mechanical cue that stimulated the chondrogenic differentiation of rabbit MSCs cultured in the alginate layer, without exogenously added growth factors such as TGF-β. Therefore, this cell exciter system should be useful for many potential applications toward chondrogenesis of MSCs.

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