Fabrication and Test of a Cell Exciter Actuated by an Electromagnetic Force for the Chondrogenic Differentiation of Mesenchymal Stem Cells

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Abstract - This paper presents the fabrication and test of a micro cell exciter actuated by an electromagnetic force for the study on the chondrogenic differentiation of rabbit mesenchymal stem cells (MSCs). The micro cell exciter is designed to apply compressive loading to the alginate gel mixed with the MSCs. The magnetic cell exciter consists of an actuator component and a cartridge-type chamber component. An actuator is composed of a permanent magnet, a core and a coil. The chamber has seven PMMA wells and a cell culture Petri dish. Two types of alginate gels were stimulated by the cell exciters for 10 minutes every 12 hours for 7 days. In order to determine the expression of these matrix components during differentiation, RT-PCR analysis was performed. Collagen type II was expressed in the MSCs subjected to the compressive stimulation.

Keywords: Cell Exciter, Cell Differentiation, Cell Exciter, Chondrogenesis, Mesenchymal stem cells (MSCs)

1. Introduction

Stem cells have remarkable potential to develop into many different cell types in the body. Therefore, they can be applied to the medical therapies associated with bone marrows, nerve cells, heart muscle cells and pancreatic islet cells [1].

Among the stem cells, bone marrow-derived mesenchymal stem cells (MSCs) attract worldwide attention as a source of easy isolation [2], regenerative potential [3], and genetic plasticity [4, 11]. Previous studies have shown that MSCs reproducibly and predictably differentiate into bone [5, 6], cartilage [7-9], adipose [5], and other tissues [4, 5] both in vitro and in vivo. The differentiation into cartilage from MSCs can be applied to develop a replacement therapy for damaged or diseased cartilage.

MSCs require appropriate signals to differentiate specifically into cartilage. The biochemical factors have been investigated to determine the mechanisms of differentiation of stem cells [6, 7]. Recently, several groups reported that physical and mechanical stimulations play another important role to differentiate MSCs [9, 10]. However, these methods of traditional experimentation have several drawbacks, such as the large quantity of sample, low reliability, and low reproducibility.

Compared with the previous researches, the micro cell exciter of this paper has two key merits; reduction of the necessary quantity of MSCs and increment of the reliability concerning partitioning and quantification of MSCs. Several types of stimulation are tested to identify the effective stimulation in the chondrogenic differentiation of MSCs. Histological assay and reverse transcriptase (RT)-PCR analysis were performed to observe the expression of the chondrogenesis.

2. Micro Cell Exciter

Fig. 1 shows the structure of the micro cell exciter actuated by the electromagnetic force. It consists of an actuator component and a cartridge-type chamber component. The actuator consists of seven magnetic actuators. Each actuator is composed of a permanent magnet, a core and a coil. The chamber contains seven wells, fabricated with poly methylmethacrylate (PMMA) and a cell culture Petri dish. The size of each well is 12 mm in diameter and 5 mm in height. Fig. 2 shows the photograph of the fabricated cell exciter.

With the chamber part on the actuator part, the 3-demensional alginate gels including MSCs are placed on the wells. The chondrogenic medium is then filled up in each chamber. The metal caps cover alginate gel within the chondrogenic medium. When the input voltage is applied to an actuator coil, the electromagnetic force attracts the metal cap and the metal cap compresses MSCs.

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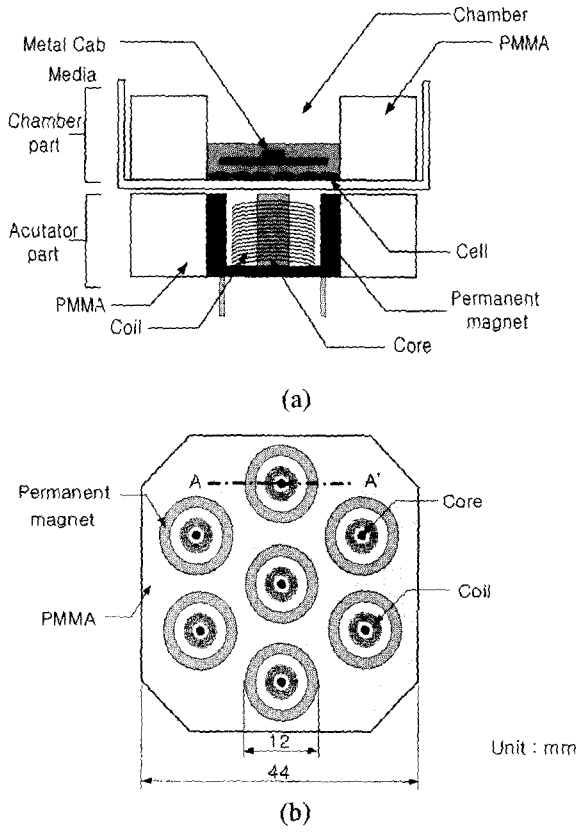


Fig. 1 Structure of the micro cell exciter actuated by electromagnetic force. (a) Cross-sectional view of exciters. (b) Top view of the actuator.

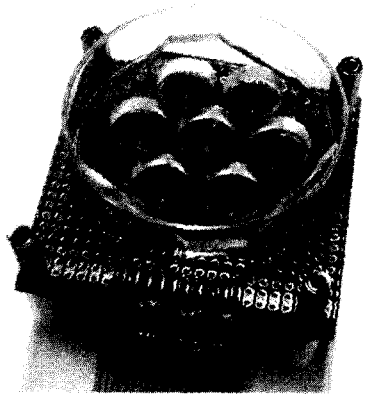


Fig. 2 Photograph of the fabricated cell exciter.

3. Methods

Rabbit MSCs were isolated from rabbit bone marrow and cultured in a 3-dimensional environment such as the bead-type alginate and disk-type alginate.

3.1 Cell isolation and expansion

Bone marrow from the knees of 2-week-old rabbits being slaughtered was aspirated into syringes including 40mL PBS solution and antibiotics. The bone marrow samples were washed with phosphate buffered saline (PBS) and centrifuged at 1700 rpm for 10 min in mesenchymal stem cell growth medium (10% of newborn calf serum (NCS), 1% α-MEM) before being seeded in alginate beads at a density of 2×10^6 cells/mL of alginate. The number of mononuclear cells was determined with a hemocytometer and plated in a 150mm patri-dish at the density of approximately 1.5×10^7 cells/150mm dish. After 6 days, MSCs were dissociated from dishes with 0.05% trypsin- ethylenediaminetetra-acetic acid (EDTA). The trypsinized MSCs were centrifuged and re-suspended in alginate gel described as below.

3.2 Alginate gel formation and in vitro cultivation

The alginate beads that contained cells were constructed with two types as shown in Fig. 3. The bead-type alginate gel was formed as follows. MSCs were mixed with 2% alginate solution. The suspension was transferred into a 10-mL sterile syringe and expressed drop by drop through a 23-gauge needle into the solution of 102 mM $CaCl_2$. Then, gelation of alginate beads occurred instantaneously. The average bead height was 2 mm. Following instantaneous gelation, the beads were polymerized for a period of 10 min in a $CaCl_2$ solution.

The disk-type alginate gel was formed as follows. The MSCs were mixed with 2% alginate solution. Then, the well-mixed solution was quickly spread into inner cell plates. These plates were immersed in a solution of 102mM $CaCl_2$. After several minutes, the alginate gel was separated from the plates.

The alginate gel that was immersed in the chondrogenic medium was incubated at 37°C in 5% CO_2 environment. The chondrogenic medium consists of high glucose Dulbecco's Modified Eagle Medium (DMEM), 1% peniscilin streptomysin, ITS, 50 μg/mL ascorbic acid 2-phosphate, 100nM dexamethasone, 40 μg/mL praline, 1.25 mg/mL bovine serum albumin (BSA). Culture medium on each chamber was replaced every 48 hours.

4. Stimulations

Two types of alginates were stimulated by cell exciter for 10 minutes every 12 hours for 7 consecutive days to determine the effect of dynamic pressure in stem cell differentiation. The voltage and frequency of the square wave applied to the exciter was 10V and 1Hz, respectively. The pressure applied to the alginate gel with a metal cap on is 35Pa when the voltage applied to the exciter is 10 V.

Table 1 presents the condition of the stimulation. There

are five groups. The first control group is not influenced by any stimulation. The second and the fourth test groups are laid on the exciter without any input signal (i.e. static magnetic field). The fifth group is stimulated by an exciter with an input signal and a metal cap (i.e. dynamic magnetic field and pressure).

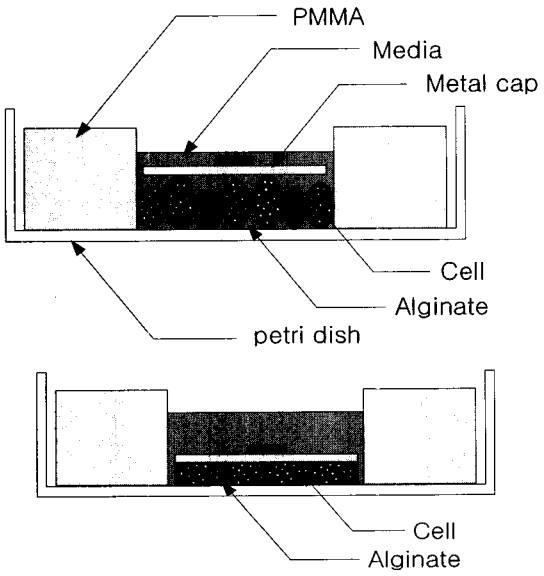


Fig. 3 The experimental setup for cell stimulation. (a) bead-type alginate gel. (b) disk-type alginate gel.

Table 1 Conditions of stimulation.

(MF: magnetic field, PR: pressure)

Stimulation \ Conditions		Permanent magnet	Voltage applied	With a metal cap
1	None	no	no	no
2	Static MF	yes	no	no
3	Dynamic MF	yes	yes	no
4	Static MF and Static PR	yes	no	yes
5	Dynamic MF and Dynamic PR	yes	yes	yes

5. Analysis

We analyzed the chondrogenic differentiation of MSCs by means of Histology and RT-PCR.

5.1 Histology

At the end of the culture period, the test groups and control group were harvested for histological studies. The alginate gels were fixed overnight in 4% paraformaldehyde at 4°C and transferred to 70% ethanol until embedded in paraffin according to standard histological techniques. Sections were stained with hematoxylin and eosin (H&E).

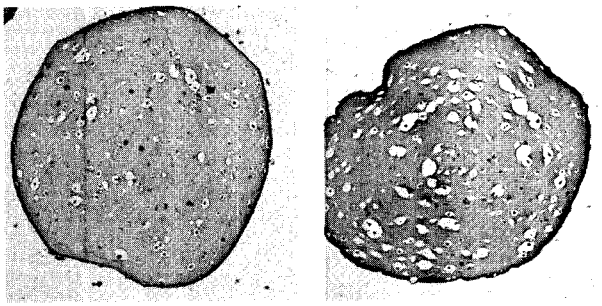
5.2 RNA preparation and RT-PCR

Total RNA was prepared from undifferentiated MSCs and from the disk-type alginate gel cultured for 7 days using the Qiagen RNeasy mini kit. DNasetreated RNA was used for RT-PCR with the Super Script Preamplification System (Gibco BRL) and random priming of the RNA. cDNA (0.1 mg) was amplified in a Perkin–Elmer 9500 PCR machine using the primers at a final concentration of 400 nM in PCR SuperMix (Life Technologies) in a final reaction volume of 25 mL. An annealing temperature of 57°C was used. PCR products were visualized on 1% agarose gels containing 0.1 mg/mL ethidium bromide using ultraviolet light. The identity of each targeted PCR amplification product was confirmed by DNA sequence analysis of agarose gel-purified bands (Promega Wizard PCR Preps DNA Purification System). The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a control and normalization of the loading volumes to produce equal levels of GAPDH expression.

6. Results

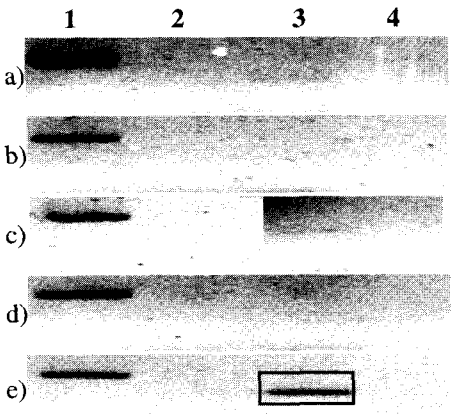
The MSCs morphology in bead-type was observed by histological assay and H&E (hematoxyline & eosin) staining. The samples were harvested and examined grossly at 7 days. The gross examination appeared similar in size in all groups. Fig. 5 shows the result of the experiment with bead-type alginates. The sizes of lacunae of the test group are two times larger than that of the control group. Lacunae are a specific phenomenon of the chondrogenic differentiation.

The MSCs in disk-type were analyzed by RT-PCR (reverse transcriptase – polymerase chain reaction) for examination of collagen type II as the specific marker of chondrogenic differentiation of MSCs. Fig. 6 shows the result of the experiment with disk-type alginates. The expression of collagen type II was observed only in the sample stimulated with dynamic magnetic field and dynamic pressure.



(a) (b)

Fig. 5 Analysis of histology (H&E). (a) The control group. (no stimulation) (b) The fifth test group. (dynamic MF and PR stimulation)



1: GAPDH, 2: Aggrecan, 3: Collagen type II, 4: SOX9

Fig. 6 Analysis by RT-PCR. (a) Bare condition, (b) Static MF condition, (c) Dynamic MF condition, (d) Static MF and static PR condition, (e) Dynamic MF and dynamic PR condition.

The results show the feasibility of electromagnetic cell exciter as an effective test tool and illustrate that mechanical stimulation is one of the important factors for the differentiation of MSCs.

7. Conclusion

MSCs are the cells at the early stage of differentiation and are metabolically active for the differentiation of cellular morphology and protein synthesis. Therefore we hypothesized that MSCs are capable of responding to physical stimuli such as cell exciters. In this research, type II collagen was observed in the mechanical stimulus group as compared with the control group.

The mechanisms that induce this partial differentiation are unclear. However, the results of this study indicate that the physiologic dynamic stimulation within the appropriate ranges of the voltage and frequency is effective to differentiate MSCs. These stimuli may be a useful tool for functional tissue engineering.

Recreating the *in vitro* mechanical environment for the differentiation of MSCs in the field of tissue engineering may require not only mechanical stimulation but also the administration of growth factors. In our future work, we will investigate the effect of the growth factor in addition to the mechanical stimulation and find the optimal stimulation for the chondrogenic differentiation.

Acknowledgement

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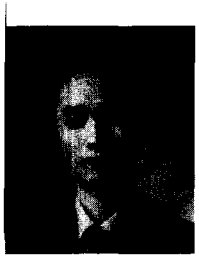

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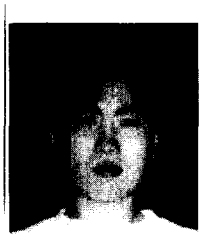

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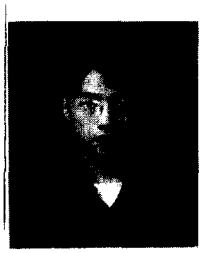

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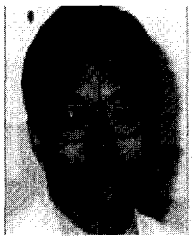

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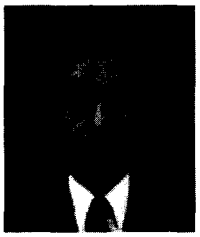

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