Our goal is the efficient fabrication of the desired clinical substitutes for smooth muscle tissues in vitro, holding the capacity for contraction similar to the native smooth muscle tissues and being stably located after transplantation. In this study, functional smooth muscle constructs were organized in a bioreactor using prealigned and differentiated SMCs. They were well proliferated after a 7-day culture period in a bioreactor, retaining premodulated contractile characteristics. As shown in the comparison of their mechanical properties, the premodulated constructs showed malleable and durable properties comparable to native tissues in the elastic deformation region.

REFERENCES


Low-intensity Ultrasound Stimulation Enhances Chondrogenic Differentiation in Alginate Culture of Mesenchymal Stem Cells

*Hyun Jung Lee, †Byung Hyune Choi, *‡Byoung-Hyun Min, §Young Sook Son, and †¶So Ra Park

*Department of Biomedical Engineering, Ajou University, Suwon; †Research Institute for Medical Sciences, Inha University College of Medicine, Incheon; ‡Department of Orthopedic Surgery, Ajou University, School of Medicine, Suwon; §Laboratory of Tissue Engineering, Korea Cancer Center Hospital, KAERI, Seoul; and †¶Department of Physiology, Inha University College of Medicine, Incheon, Korea

Abstract: Mesenchymal stem cells (MSCs) are regarded as a potential autologous source for cartilage repair, because they can differentiate into chondrocytes by transforming growth factor-beta (TGF-β) treatment under the 3-dimensional (3-D) culture condition. However, more efficient and versatile methods for chondrogenic differentiation of MSCs are still in demand for its clinical application. Recently, low-intensity ultrasound (LIUS) was shown to enhance fracture healing in vitro and induce chondrogenesis of MSCs in vitro. In this study, we investigated the effects of LIUS on the chondrogenesis of rabbit MSCs (rMSCs) in a 3-D alginate culture and on the maintenance of chondrogenic phenotypes after replating them on a monolayer culture. The LIUS treatment of rMSCs increased: (i) the matrix formation; (ii) the expression of chondrogenic markers such as collagen type II, aggrecan, and Sox-9; (iii) the expression of tissue inhibitor of metalloprotease-2 implicated in the integrity of cartilage matrix; and (iv) the capacity to maintain the chondrogenic phenotypes in a monolayer culture. Notably, LIUS effects were clearly shown even without TGF-β treatment. These results suggest that LIUS treatment could be an efficient and cost-effective method to induce chondrogenic differentiation of MSCs in vitro for cartilage tissue engineering. Key Words: Low-intensity ultrasound—Chondrogenesis—Mesenchymal stem cells—Alginate culture.

Mesenchymal stem cells (MSCs) are undifferentiated pluripotent cells capable of differentiating into many cell types such as osteoblasts, chondrocytes, adipocytes, and myocytes (1). Previous studies showed that MSCs derived from bone marrow and perichondrium express markers for chondrogenesis such as aggrecan and collagen type II have a chondrogenic potential (2,3). Furthermore, MSCs...
implanted into surgically created osteochondral defects in rabbits are able to differentiate into chondrocytes and improve cartilaginous tissue repair (4).

Many factors including transforming growth factor-beta (TGF-β), bone morphogenetic proteins, and fibroblast growth factor-2 (FGF-2) are known to affect the chondrogenic differentiation of MSCs (5,6). Among them, TGF-β is known as a critical factor for initiation of chondrogenic differentiation in vitro and in vivo (5,7). TGF-β1 was used originally in the chondrogenic differentiation of chondrocytes and MSCs (2,8), but TGF-β2 or TGF-β3 were also shown to be effective in MSCs (5,9,10). In addition to the protein factors, mechanical factors such as cyclic compressive loading and three-dimensional (3-D) culture system are also known to induce chondrogenesis of MSCs in vitro (2,3,11–13).

Low-intensity ultrasound (LIUS) is well known to improve fracture healing and increase the mechanical strength of fracture callus in clinical studies and animal models (14,15). LIUS is also reported to enhance repair of articular cartilage in animal model of cartilage defect (16,17). In cultured chondrocytes in vitro, LIUS stimulation enhances the synthesis of matrix proteins such as collagen type II and proteoglycans depending on the culture condition and LIUS intensity (18–22).

Considering the extensive studies on the chondrogenic differentiation of MSCs so far, the effect of LIUS on the process is relatively poorly investigated. Recently, Ebisawa et al. showed that the LIUS treatment enhanced the TGF-β-mediated aggrecan deposition in pellet culture of human MSC (10). However, no further details on LIUS effects such as the changes in the expression of collagen type II and other related proteins were suggested in their experiment. In this study, we investigated the effect of LIUS on the expression of chondrogenic markers and genes involved in matrix degradation during the chondrogenic differentiation of rabbit MSCs (rMSCs) in alginate culture both at the transcriptional and protein levels. The effect of LIUS was also investigated on the maintenance of chondrogenic phenotypes after replating the differentiated cells from the alginate culture on a two-dimensional plate.

**MATERIALS AND METHODS**

**Isolation and culture of bone marrow MSCs**

MSCs were isolated from 2-week-old female New Zealand white rabbits (Joong-Ang Experimental Animal Center, Seoul, Korea). Immediately after sacrificing the rabbits with ketamine and ethyl ether, bone marrow aspirates were obtained aseptically from the tibia and femur by flushing several times with phosphate-buffered saline (PBS) (23). The aspirates were then suspended in 5% acetic acid in PBS to remove the red blood cells, and total mononuclear cells (MNCs) were recovered by centrifugation at 1500 g for 15 min (7). The cell pellets (MNCs) were resuspended in an α-minimum essential medium (α-MEM, Sigma, St. Louis, MO, USA) with 10% fetal bovine serum (FBS). The MNCs were then plated (1.5 × 10^7 cells) on a 150-mm dish and incubated at 37°C under 5% CO₂. After 6 days, the culture was replenished with fresh medium to remove nonadherent cells and the adherent MSCs were maintained in the same medium (24). At day 14 from the initial plating, the MSCs were passaged by trypsin treatment (passage 1). After 1 week, the cells at passage 2 were used for 3-D alginate culture and LIUS treatment.

**Alginate culture**

When the secondary culture of MSCs (passage 2) was about 80% confluent, cells were trypsinized and suspended in a 2% alginate (Sigma) solution at a density of 2 × 10^6 cells/mL. The cell/alginate mixture was slowly dropped from a 23-gauge needle into 102 mM CaCl₂ solution, where CaCl₂ encapsulates the alginate/cell complex immediately upon contact to form spherical beads. After 10 min, the beads were rinsed four times with 0.15 M NaCl solution to remove CaCl₂ and once with serum-free chondrogenic defined medium (high-glucose Dulbecco's modified Eagle's medium [DMEM], ITS supplement (1.0 mg/mL insulin from bovine pancreas, 0.55 mg/mL human transferrin, and 0.5 μg/mL sodium selenite), 50 μg/mL of ascorbic acid 2-phosphate, 100 nM dexamethasone, 40 μg/mL of proline, 1.25 mg/mL of bovine serum albumin [BSA], and 100 μg/mL of sodium pyruvate; all from Sigma) (25). The alginate beads were then moved to a 60-mm culture dish, and overlaid with serum-free chondrogenic defined medium for treatment.

**Treatment of ultrasound and TGF-β3**

The alginate cultures were treated with 10 ng/mL of TGF-β3 (PeproTech, London, UK) and/or LIUS for 1 or 2 weeks. TGF-β3 was used instead of TGF-β1 because it was previously shown to have better activity to induce chondrogenic differentiation of MSCs (9). The LIUS stimulation was performed as described previously with some modifications (21). Briefly, LIUS was treated for 10 min every 12 h for 1 or 2 weeks at a frequency of 1 MHz and an intensity of 200 mW/cm² in a continuous-wave fashion. The intensity of 200 mW/cm² was selected based on our data using chondrocytes (22, unpublished data) and
a preliminary result using MSCs (Fig. 1). The equipment used was Noblelife (Duplogen, Suwon, Korea) that has three 5-cm-wide transducers and controllers for the intensity and treatment time. After treatment, the cultures were incubated in a humidified incubator at 37°C under 5% CO2 for 1 or 2 weeks, with the culture medium changed twice per week.

**Analysis of chondrogenic phenotypes in a monolayer culture**

After LIUS treatment, the alginate beads were washed twice with 0.15 M NaCl and incubated at 37°C for 15 min after the addition of three volumes of 55 mM sodium citrate solution in order to dissolve the beads for cell recovery. The cells were harvested by centrifugation at 1500 g for 5 min and replated on a monolayer culture in DMEM (high-glucose DMEM, Gibco BRL, Grand Island, NY, USA) supplemented with 10% FBS. When the cells (passage 0, P0) were at near confluence, they were detached by trypsin treatment and replated onto 60-mm dishes (the first passage, P1). The cells were repeatedly passaged at confluence with time intervals of about 7 days between passages (P2–P4). Some of the cells at each passage were spared for examining the doubling time by cell counting after trypan blue staining and the expression of chondrogenic markers of Sox-9, aggrecan and collagen type II by reverse transcription polymerase chain reaction (RT-PCR) analysis.

**RT-PCR**

Total RNA was extracted from cells in alginate beads or in monolayer culture using Trizol reagent (Gibco BRL). A total RNA of 2 µg was used for cDNA synthesis using Superscript first-strand synthesis system (Gibco BRL) and 2 µL of the synthesized cDNA was used for polymerase chain reaction using primers listed in Table 1; 18S rRNA was used as a control. To exclude a possibility of genomic DNA contamination, RT-PCR was also carried out using total RNA without reverse transcriptase. The PCR products were analyzed on a 1.5% agarose gel.

**Western blot analysis**

Cell extracts were prepared using a lysis buffer (40 mM Tris-HCl, pH 8.0, 120 mM NaCl, 0.5% NP-40, 100 µg/mL of phenylmethanesulfonyl fluoride, and 2 µg/mL each of aprotinin, pepstatin, and leupeptin). Total amount of proteins was determined using the bicinchoninic acid (BCA) assay (Sigma). The proteins were separated on an 8% sodium dodecyl sulfate–polyacrylamide gel and transferred onto nitrocellulose membranes (Protran, Schleicher and Schuell, Keene, NH, USA). After blocking, the blots were incubated with primary antibodies to collagen type II, tissue inhibitor of metalloprotease (TIMP)-2, or matrix metalloprotease (MMP)-3 overnight at 4°C. The blots were then incubated with sheep antimouse immunoglobulin G (IgG) conjugated with horseradish peroxidase for 1 h at room temperature. Immunoreactive signals were detected using the enhanced chemiluminescence (ECL) kit (Amer- sham, Piscataway, NJ, USA). All antibodies were purchased from Chemicon (Temecula, CA, USA).

**Histological and immunohistochemical evaluation of alginate cultures**

After LIUS stimulation, the alginate beads were rinsed in PBS and polymerized in 20 mM BaCl2 solution. The samples were then fixed for 1 h in 4% formaldehyde, dehydrated, and embedded in paraffin wax. Sections of 5-µm-thick were cut through the embedded beads and stained with Safranin O/fast green for histological analysis.

Some sections were immunostained for collagen type II protein. Briefly, the sections were washed sequentially in 70% ethanol for 10 min and in PBS for 5 min, and treated with 3% H2O2 in PBS for 5 min to inactivate endogenous peroxidase. Then, they were permeabilized with 0.15% Triton X-100 in PBS and blocked with 1% BSA for 30 min. Monoclonal anti-

**TABLE 1. Sequence of the primers used in RT-PCR**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>18S rRNA</td>
<td>Sense</td>
<td>5’-caaccttcagagggacaag-3’</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>3’-ccccacaatttaaagcaaan-5’</td>
</tr>
<tr>
<td>Sox-9</td>
<td>Sense</td>
<td>5’-ctacacatcatacttagtag-3’</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>3’-cagcagatcagctgct-5’</td>
</tr>
<tr>
<td>Aggrecan</td>
<td>Sense</td>
<td>5’-gtaattttagttttagttttag-3’</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>3’-gatgagatgagatgagatg-5’</td>
</tr>
<tr>
<td>Type II collagen</td>
<td>Sense</td>
<td>5’-gtagatttctctctctctctct-3’</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>3’-tagatttctctctctctctct-5’</td>
</tr>
</tbody>
</table>
body to human collagen type II (1:500, Chemicon) were treated for 1 h at room temperature. The signals were detected with a biotinylated antimouse secondary antibody and peroxidase-conjugated avidin system according to the manufacturer’s instruction (Vector Laboratories, Burlingame, CA, USA). The immunostained sections were counterstained with Mayer’s hematoxylin (Sigma) before microscopic examination (Nikon E600, Nikon, Tokyo, Japan).

**Statistical analysis**

Statistical difference in the mechanical and biochemical assays were sought between groups using a one-way analysis of variance, a Student–Newman–Keuls test, and Student’s t-test (two-tail). A statistical significance was assigned as *P < 0.05, **P < 0.01, and ***P < 0.001.

**RESULTS**

rMSCs were isolated and cultured in monolayer, which exhibited a fibroblast-like morphology as reported previously (24). Then, the cells were transferred to alginate culture at the time of second passage and treated with LIUS and TGF-β3 for 1 or 2 weeks as described in Fig. 2A. At 1 week after TGF-β3 treatment alone, the cells were observed to enter the chondrogenic pathway with Safranin-O (a matrix marker) positive lacunae around the cells (Fig. 3A) and the expression of collagen type II (Fig. 4A) as expected. At 2 weeks, they were also positive for the chondrogenic markers and grew as small aggregates with a round or ellipsoidal morphology. In contrast, the control cells without any treatment showed no sign of chondrogenic differentiation both at 1 and 2 weeks. Notably, the cells treated with LIUS showed the Safranin-O positive lacunae and expression of type II collagen even without TGF-β3 treatment, and cotreatment of LIUS and TGF-β3 showed slight additive effects (Figs. 3B and 4B). These results suggest that LIUS can induce chondrogenic differentiation of MSCs in alginate culture.

To further support the chondrogenic induction of MSCs by LIUS, the expression of genes involved in chondrogenic differentiation was analyzed by RT-PCR analysis. The RT-PCR was performed for Sox-9, type II collagen, and aggrecan using specific primers (Table 1). Sox-9 is a key transcription factor.
in the chondrogenic differentiation. Its mRNA was detected to some extent even without any treatment in alginate culture from 1 week and was induced clearly by LIUS or TGF-β3 alone (Fig. 5). The cotreatment of LIUS and TGF-β3 showed a slight additive effect on the Sox-9 expression particularly at 2 weeks. The mRNA level of type II collagen was very low without any treatment, but induced by the treatment of TGF-β3 alone at 2 weeks or LIUS alone both at 1 and 2 weeks (Fig. 5). Cotreatment of them showed no additive effects on the type II collagen expression. Although TGF-β3 alone at 1 week showed only a slight activity in this set of experiment, we could repeatedly observe its effect in other sets. The mRNA level of aggrecan at 1 week was very low without any treatment (Fig. 5); however, it was clearly induced by either or cotreatment of LIUS and TGF-β3 at 1 week. At 2 weeks, the mRNA level of aggrecan appeared already high in alginate culture without any treatment and only a slight induction was observed by LIUS.

We further examined the effect of LIUS on the expression of type II collagen, TIMPs, and MMPs by Western blot analysis. The balance of TIMPs and MMPs is known to be important in maintaining the integrity of cartilage matrix. The protein level of type II collagen was also induced by LIUS and TGF-β3 (Fig. 6) early from 1 week, which was consistent with the RT-PCR result. When the protein level of TIMP-2 was examined, it was induced by LIUS but only with cotreatment of TGF-β3 both at 1 and 2 weeks (Fig. 6). In contrast, the level of MMP-3 was not induced by any of the treatments.

We then examined the effect of LIUS on the maintenance of the chondrogenic phenotypes during the passages, once the differentiated cells in alginate culture were replated on a monolayer culture. The alginate culture was left untreated or treated with LIUS alone for 1 week during the chondrogenic induction. Then, cells were recovered out of the beads and cultured in a monolayer condition for several passages (Fig. 2B). When examined by the doubling time, the proliferative activity of cells was initially relatively weak (P0 cells) but recovered soon from the next passage (P1) and gradually decreased again by the passages (Table 2). The LIUS-treated group showed a similar pattern of but quite higher
proliferative activity than the untreated control until the passage 2 (P2) cells.

The levels of chondrogenic markers of Sox-9, aggrecan, and type II collagen mRNAs were also decreasing overall by the passages, similar to the pattern of the doubling time in Table 2 (Fig. 7). The level of Sox-9 mRNA was somewhat increased and that of aggrecan mRNA was relatively unchanged at the early passages (P1 and P2), but they were decreased rapidly after that. The level of type II collagen was decreased gradually from the P0. The LIUS-treated group showed clearly higher levels of their expression at early passages, which became similar to the levels of the control group after passages 2–3.

**DISCUSSION**

Natural cartilage remodels both in vivo and in vitro in response to mechanical forces and hence, mechanical stimulation is believed to have a potential as a tool to modulate extracellular matrix synthesis in cartilage formation. Fluid-induced shear is known to enhance chondrogenesis of human cartilage cells. The LIUS treatment resulted in higher expression levels of Sox-9 and aggrecan at early passages (P1 and P2), which were comparable to those of the control group after passages 2–3. This suggests that LIUS may have a potential for cartilage tissue engineering.

**TABLE 2. Doubling times for cells in each passage after monolayer culture (days)**

<table>
<thead>
<tr>
<th>Cells in passages</th>
<th>Control</th>
<th>LIUS pretreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0</td>
<td>1.67</td>
<td>1.23</td>
</tr>
<tr>
<td>P1</td>
<td>1.14</td>
<td>0.78</td>
</tr>
<tr>
<td>P2</td>
<td>1.2</td>
<td>0.83</td>
</tr>
<tr>
<td>P3</td>
<td>1.5</td>
<td>1.47</td>
</tr>
<tr>
<td>P4</td>
<td>1.72</td>
<td>1.6</td>
</tr>
</tbody>
</table>

**FIG. 7.** Effects of LIUS treatment on the expression of chondrogenic markers in the monolayer culture after replating from the alginate. Experimental schemes were described in Fig. 2B. The rMSCs under chondrogenic differentiation were treated with LIUS for 1 week and replated on a monolayer culture. (A) Expression levels of Sox-9, aggrecan, and type II collagen were measured by RT-PCR analysis along with the passages (P0–P4). mRNA levels before replating were shown in the left (alginate 1w). Those of the undifferentiated rMSCs and rabbit chondrocytes (rCh) without LIUS treatment were shown as negative and positive controls, respectively, at each end of the right panel. (B) Intensities of the specific signals from P0–P4 were normalized by the levels of 18S rRNA, and the results from three independent experiments were described. The error bars represent standard deviations (SDs).

\[ n = 3, **P < 0.01. \]
chondrocytes (26), and cyclic pressure is reported to enhance the chondrogenic phenotype of mesenchymal progenitor cell aggregates (12,13). In this study, we demonstrated that LIUS stimulated chondrogenic differentiation of rabbit bone marrow MSCs in alginate culture. Differentiating chondrocytes go through an initial phase of cell condensation and a subsequent deposition of cartilage specific matrix. In the alginate culture of MSCs, LIUS induced the cartilage matrix synthesis (Figs. 3 and 4) and expression of chondrogenic markers such as aggrecan, type II collagen, and Sox-9 (Fig. 5), as well as TIMP-2 that is important in matrix integrity (Fig. 6). The effects of LIUS on the expression of chondrogenic markers were shown independently of TGF-β3 treatment, a well-known inducer of chondrogenesis. This result suggests that LIUS by itself could enhance chondrogenic differentiation of MSCs in alginate culture at least to some extent.

Similarly to the LIUS effects, cyclic pressure was also shown to induce the chondrogenic differentiation of rabbit and human MSCs from bone marrow in vitro independently of TGF-β treatment (12,13). The TGF-β signal is mediated by specific membrane receptors and Smad pathway (27). The signaling pathway of LIUS is not well understood but probably includes mechanotransduction pathways mediated by integrins (28). The mechanotransduction pathway in articular chondrocytes is known to include integrins, stretch-activated ion channels, and interleukin-1 (29). Therefore, TGF-β3 and LIUS appeared to use different signal pathways, and play independent and probably redundant roles in our experiment. However, we do not completely exclude the possibility that LIUS treatment induced the expression of TGF-β family proteins.

Chondrocytes in monolayer culture in vitro is usually believed to dedifferentiate and lose their chondrogenic phenotype along with passages. Previously, human synovial membrane MSCs differentiated into chondrocytes in pellet culture were shown to lose their chondrogenic phenotype by 24 h after replating in monolayer culture (30). In our replating experiment, the chondrogenic cells maintained well their chondrogenic phenotypes in monolayer culture until passage 2 (for 3 weeks) when pretreated with LIUS (Fig. 7). This result suggests that the LIUS treatment is effective in maintaining the expression of chondrogenic phenotypes even in monolayer culture. However, we do not exclude the possibility that the differences in the results were caused by differences in cell sources (human MSCs vs. rMSCs) and culture condition (pellet culture vs. aliginate culture).

The LIUS treatment was shown to have an effect on the proliferation of fibroblasts, osteoblast, and monocyte (28,31), but not in chondrocytes (26) and monolayer cultures (18,19). In our experiment, however, the pretreatment of LIUS reduced the time to population doubling of the replated cells in monolayer culture until passage 2, when compared to the untreated control (Table 2). This result is also distinct from the previous report that human bone marrow MSCs that underwent chondrogenic differentiation in alginate culture showed no significant recovery in proliferating capacity after replating in monolayer culture (3). Therefore, the LIUS pretreatment seems to be helpful not only in enhancing the proliferation capacity but also in maintaining the phenotypes of the chondrogenic cells in monolayer culture.

Sox-9 is a key transcription factor required for cartilage formation (32), and its expression was shown to precede the up-regulation of cartilage-specific genes in vitro chondrogenesis (33). In this report, we could observe the expression of Sox-9 even before any treatments in alginate culture, and it was further induced by LIUS treatment (Fig. 5). This result suggests that its expression is also influenced by other factors such as 3-D alginate culture as described previously (2,3,8,11). The early expression of Sox-9 without the type II collagen and aggrecan expression may imply that either its level at that point was not sufficient or other transcription factor(s) are also necessary to induce their expression.

MMPs are implicated in the degradation of cartilage matrix in the context of arthritis, and TIMPs play a role in the maintenance of matrix integrity by inhibiting MMP activity (34,35). Therefore, regulation of the balance between MMPs and TIMPs could be of great therapeutic value for the treatment of patients with arthritis and cartilage tissue engineering (36). In this study, LIUS stimulated the TIMP-2 expression in alginate culture when cotreated with TGF-β3, but showed no effect on the expression of MMP-3 (Fig. 6). It is preliminary to make a conclusion, but this result suggests that LIUS could inhibit the cartilage matrix turnover by augmenting the TIMP expression, thereby being a useful tool in cartilage tissue engineering. However, the LIUS effect on TIMPs appears not to be the cause of the LIUS-enhanced chondrogenic differentiation of MSCs, because it was not observed without cotreatment of TGF-β3.

Our observation that LIUS induced the chondrogenic differentiation of MSCs without TGF-β3 treatment is not in agreement with a recent report that LIUS enhanced chondrocyte differentiation of human MSCs only in TGF-β3-treated pellets (10).
speculate that the discrepancy between the results could be caused by differences in (i) species (ii), culture methods, or (iii) treatment conditions. We used an alginate culture of MSCs from rabbit, while they used a pellet culture of human MSCs (see below for details). Besides, they treated the culture with LIUS every day for 20 min at an average intensity of up to 120 mW/cm² and at a frequency of 1.0 MHz with a 200-µs tone burst repeating at 1.0 KHz, which is somewhat different from our condition (see the Materials and Methods section).

A particular culture condition such as the 3-D culture system is believed to require factors to induce and maintain the chondrogenic phenotype of MSCs or chondrocytes in vitro (2,3,8,11). The pellet culture system has been the most widely used method for the chondrogenesis of MSCs (2); however, it is technically difficult to get homogeneously differentiated pellets and has some disadvantages such as a limited scale of individual pellets for molecular biological analysis. Recently, the alginate culture system was reported to be a relevant model for the study of chondrogenic differentiation of MSCs (3,11,37). For this reason, we used the alginate culture system of MSCs to study the LIUS effects in this study. In the parallel experiments using monolayer culture of MSCs, we actually could not observe the LIUS effects shown in alginate culture on the expression of type II collagen and aggrecan (data not shown). This result suggests that the alginate culture system is essential for the induction of chondrogenic differentiation of MSCs at least by LIUS.

MSCs from bone marrow are highly potent to chondrogenic differentiation, which was efficiently enhanced by LIUS stimulation. We think that the stem/progenitor cells from other sources could respond to LIUS stimulation differently depending on their chondrogenic potential. In a patient-matched comparison, for example, adipose tissue-derived progenitor cells were shown to be less potent in chondrogenic differentiation than patient-patching bone marrow-derived progenitor cells (38), and possibly, the LIUS activity could be less effective. Likewise, the chondrogenic differentiation of muscle-derived stem cells, shown to have both myogenic and osteogenic differentiation potentials (39), could be enhanced by LIUS depending on their chondrogenic potential. The effect of LIUS on the differentiation of stem/progenitor cells into other lineages than chondrocytes is not clear, but could largely depend on their response to mechanical stimulation. In this aspect, osteogenic differentiation of osteoblasts was also shown to be enhanced by LIUS in vitro (40).

CONCLUSION

The present study has shown that LIUS can stimulate chondrogenic differentiation of rabbit MSCs cultured on alginate beads without TGF-β treatment. Therefore, LIUS as a chondrogenic mechanical stimulus would promise many useful applications for cartilage repair by enhancing the efficiency and/or removing the need for TGF-β during the chondrogenesis of MSCs in vitro.

Acknowledgment: This study was supported by a grant of the Korea Health 21 R & D Project, Ministry of Health & Welfare, Republic of Korea (0405-BO01-0204-0006).

REFERENCES


---

**Immobilized Microagal Cells as an Oxygen Supply System for Encapsulated Pancreatic Islets: A Feasibility Study**


*Diabetes and Obesity Research Laboratory, Felsenstein Medical Research Center, Sackler Faculty of Medicine, Tel-Aviv University, Bellinson Campus, Petah Tikva; ‡Department of Plant Sciences, Tel-Aviv University, Tel-Aviv; and §Beta-O2 Technologies Ltd, Petach Tikva, Israel*

**Abstract:** Recently, a novel technique for oxygen supply to immunoisolated islets, which adopts the photosynthetic capacity of microalgae to generate oxygen, has been described. Illuminated algal cells, co-immobilized with islets in one compartment, were capable of restoring glucose-stimulated insulin secretion during perfusion with anoxic medium. In the present study, a new model system for photosynthetic oxygen supply to encapsulated islets, containing two separate compartments—one for oxygen-producing alga cells and the other for insulin-secreting pancreatic islets—is described. No insulin response to increasing glucose concentrations was found when encapsulated islets alone were perfused with oxygen-free medium. However, when the perfused chamber contained not only encapsulated islets, but also illuminated algae, immobilized in alginate, the islets showed twice the amount of insulin.

Received December 2005; revised March 2006.

Address correspondence and reprint requests to Dr. Konstantin Bloch, Diabetes and Obesity Research Laboratory, Felsenstein Medical Research Center, Rabin Medical Center, Bellinson Campus, 49100 Petah Tikva, Israel. E-mail: kbloch@post.tau.ac.il

*Artif Organs, Vol. 30, No. 9, 2006*