Chondrogenesis of human mesenchymal stem cells encapsulated in alginate beads

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Abstract: Mesenchymal stem cells (MSCs) have the capacity for self-renewal and can form bone, fat, and cartilage. Alginate forms a viscous solution when dissolved in 0.9% saline and gels on contact with divalent cations. The viability and phenotype maintenance of chondrocytes in alginate beads have been well documented. However, little is known about the effect of microencapsulation in alginate on chondrogenesis of MSCs. In this study, human MSCs encapsulated in alginate beads were cultured in serum-free medium with the addition of transforming growth factor (TGF)β1 (10 ng/mL), dexamethasone (10^{-7} *M*), and ascorbate 2-phosphate (50 μg/mL). The MSCs in alginate assumed a rounded morphology with lacunae around them after 1 week in culture. Cell aggregates were observed at 2 weeks or longer in culture. Histological findings agreed with the clinical determination of hyaline cartilage, characterized by isolated cells with ground substance positive in Safranin-O staining and immunohistochemistry for collagen type II at the periphery of cells. Reverse transcriptase–polymerase chain reaction (RT-PCR) revealed the expression of COL2A1 and COL10A1, marker of chondrocytes and hypertrophy chondrocytes, respectively. These results indicate MSCs in alginate can form cartilage and the MSCs-alginate system represents a relevant model for the study of the molecular mechanisms involved in the chondrogenesis and endochondral ossification. © 2002 Wiley Periodicals, Inc. J Biomed Mater Res 64A: 273–281, 2003

Key words: alginate beads; biomaterials; chondrogenesis; *in vitro;* mesenchymal stem cells (MSCs)

INTRODUCTION

Articular cartilage differs from other tissues in distributing loading forces during joint motion with very little friction and wear. The limited capacity of adult articular cartilage for self-repair has been well recognized. Many efforts have been undertaken to repair articular cartilage lesions by numerous techniques, including microfracture,[1] osteochondral allograft,[2,3] and periosteal or perichondral patches.[4–6] The main problem inherent to each of these methods is an inability to reproduce reparative tissue characteristics of hyaline cartilage for optimal filling of the defects.

More recently, tissue engineering based on cells, or on cells embedded in a suitable delivery substance, has been developed to aid in the repair of articular cartilage defects. The suitability of agarose,[7,8] collagen,[9–12] fibrin glue,[13–15] hyaluronic acid,[16,17] synthetic polymers,[18,19] and alginate[20–25] as a substance for delivering chondrocytes has been evaluated. Chondrocytes cultured at high density in monolayer usually remain phenotypically stable for only a few weeks,[26] and proteoglycan synthesized in this culture system is rapidly lost from the cell-associated matrix. Several studies have now established that the entrapment of isolated chondrocytes in gel, such as agarose, collagen, and alginate, helps promote the synthesis of collagen type II and of proteoglycan, markers of the chondrocytic phenotype.[10,11,24,25]

Adult articular chondrocytes embedded in alginate gel produce de novo a matrix rich in collagens and proteoglycans,[24] even at 8 months after encapsulation.[27] Chondrocyte-calcium alginate gel constructs could generate cartilage after implantation into subcutaneous pockets of nude mice.[22] These findings sug-

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gest that calcium alginate gels would be a suitable delivery system for the encapsulation and transplantation of chondrocytes.

An alternative approach to using differentiated chondrocytes for cartilage repair is the use of progenitor stem cells. Mesenchymal stem cells (MSCs) derived from bone marrow can proliferate without differentiation and can form bone, fat, and cartilage separately under different specified conditions.[28] Thus, culture-expanded MSCs would substitute for chondrocytes as a source of cell in therapeutic application of tissue engineering for the repair of osteochondral defects. To readily attach and proliferate for regeneration of articular cartilage, MSCs need to be delivered in situ. Encapsulation system of alginate, suitable for maintaining chondrocyte phenotype, and for delivering chondrocytes, has not been well investigated regarding its effect on chondrogenesis of MSCs.

To investigate further the potential application of the delivery system in tissue engineering, the current study was conducted with the aim of using alginate beads for the chondrogenesis of human MSCs. Efforts were made to determine the *in vitro* effects of encapsulation in alginate gel on viability, proliferation, and differentiation of human MSCs.

MATERIALS AND METHODS

Isolation and culture of bone marrow mesenchymal stem cells

Five milliliters of a heparinized bone marrow aspirate taken from the iliac crest of normal donors were washed twice with phosphate-buffered saline (PBS), suspended in plating medium Dulbecco's modified Eagle's medium containing 1 g/L of glucose (DMEM-LG; Gibco) with 100 U/mL penicillin, 100 μg/mL streptomycin, 0.25 μg/mL amphotericin; and supplemented with 10% fetal bovine serum (FBS; Gibco). Cells were seeded in 100-mm petri dishes and supported in culture at 37°C with the removal of nonadherent cells 7 days later and then medium changes every 4 days. Cells were serially trypsinized and passaged at a ratio of 1:3 every 1 week.

Cell culture in alginate beads and cell recovery

At about 80% of confluence, the adherent cells were lifted with trypsin, counted, and suspended in 1.2% wt/vol alginate (low viscosity, dissolved in PBS, pH 7.4) solution and dropped from a 16-gauge needle into a solution of 120 nmol/L $CaCl_2$ [10 m*M* N-(2-hydroxyethyl) piperazine-N′-(2-ethane sulfonic acid) (HEPES), 0.01% Tween 20, pH 7.4]. The alginate/cell suspension gelled immediately on contact with the $CaCl_2$ solution, forming spherical beads. The beads remained in the $CaCl_2$ solution for 10 min to gel completely and were then rinsed three times with PBS. They were then removed into a 50-mL polypropylene tube and overlaid with culture medium consisting of serum-free DMEM-LG, 100 U/mL penicillin, 100 μg/mL streptomycin, 0.25 μg/mL amphotericin; and supplemented with ITS+ Premix (Gibco), 10 ng/mL TGF-β1 (Pepro Tech), 10^{-7} *M* dexamethasone (Sigma), and 50 μg/mL ascorbate 2-phosphate (Nacalai, Japan). The cultures were incubated at all stages in a humidified atmosphere of 5% CO_2 in air at 37°C with medium changed twice per week.

For cell recovery, the culture medium was discarded. The beads were then washed twice with PBS and incubated at 37°C for 15 min with the addition of 2 vol of 50 m*M* ethylenediamine tetraacetic acid (EDTA, Sigma), 10 m*M* HEPES (Gibco), pH 7.4. Cell pellet was harvested by centrifuging at 400 ×g for 10 min. Supernatant was removed and cell pellet was incubated at 37°C for 30 min in complete medium with 0.06% collagenase (Gibco). Cell pellet was obtained by centrifuging at 400 ×g for 10 min.

Evaluation of morphology, viability, and proliferation

Cell morphology in alginate was observed by the use of a contrast microscope (Olympus). Viability was determined by scoring the percentage of unstained cells after staining crushed alginate beads on a slide with 0.4% Trypan blue. Cell proliferation was evaluated by the analysis of cellular DNA content using CycleTEST PLUS system as described by the manufacturer (Becton Dickinson Kit No. 340242). In brief, cell pellet recovered from alginate was incubated with trypsin buffer for 10 min at room temperature, then with trypsin inhibitor and ribonuclease (RNase) for 10 min at room temperature, and finally with cold propidium iodide stain solution for 10 min in the dark on ice. The sample was analyzed with a flow cytometer using a laser adjusted to emit 500 mW at 488 nm.

Histological, histochemical, and immunohistochemical evaluation of *in vitro* cultured beads

Cells cultured in alginate beads were rinsed briefly with washing buffer (10 m*M* TRIS-HCl, 0.14 *M* NaCl, 5 m*M* $CaCl_2$, pH 7.4), fixed in 10% buffer-based formalin for 24 h on a rocking shaker, and stored in washing buffer at 4°C until polyethylene-glycol (PEG) embedding. For this, beads were dehydrated in conical 15-mL tubes with increasing concentrations of PEG 400 (Sigma, 30, 50, 70, 90, and 100%), diluted in water and adjusted to 5 m*M* $CaCl_2$ for 30 min each at room temperature on a rocking shaker. At each step, PEG was removed under gentle vacuum with a pipette and replaced with new solutions. While dehydration with PEG 400 was being performed, PEG 1000 and 1500 (Sigma) were melted at 56°C in a water bath. Melted PEG 1000 was then kept at 46°C until use. Beads were infiltrated with pure PEG

1000, then with a mixture of PEG 1000–PEG 1500 (vol/vol) at 46° for 1 h each. The bead suspension was finally poured rapidly into a plastic mold, and the blocks were stored at 4°C after hardening at room temperature. Sections (5–10 μm) were cut and laid down on poly-L-lysine–coated slides (Sigma). The slides were brought to 55°C on a warming tray to melt the PEG and to flatten the sections by dipping the slides in 70% ethanol for 2 min. After drying, slides were stored at −20°C with desiccant. Specimens were stained with hematoxylin and eosin (H&E) and Safranin-O. Immunohistochemistry was performed using a standard indirect three-step immunoperoxidase technique. The primary antibody was a murine monoclonal antibody raised against human type II collagen (MAB1330, Chemicon).

Reverse transcriptase–polymerase chain reaction

Total RNA was prepared, using the RNeasy purification system as described by the manufacturer (Qiagen), from cells released from alginate beads. Total RNA (1 μg) was reversely transcribed with Mmlv reverse transcriptase at 42°C in the presence of oligo-dT primer. The PCR was performed using specific primers designed from the published sequence of each cDNA as follows: COL2A1, sense: 5′-CCAGGACCAAAGGGACAGAAAG-3′, antisense: 5′-TTCACCAGGTTCACCAGGATTG-3′; COL10A1, sense: 5′-AGGTGCCAAAGGGGAACAAG-3′, antisense: 5′-AATCCTGGAATGCCTGGTGG-3′; β-ACTIN, sense: 5′-GCACTCTTCCAGCCTTCCTTCC-3′, antisense: 5′-TCACCTTCACCGTTCCAGTTTTT-3′. The thermal profile was 30 s at 94°C, 30 s at 56°C, 1 min at 72°C. The amplification cycles of COL2A1, COL10A1 and β-ACTIN were all 35. The expected sizes of COL2A1, COL10A,1 and β-ACTIN were 399 bp, 429 bp, and 515 bp, respectively. To exclude possible contamination of genomic DNA, PCR was also applied to reactions without RT. The amplified complementary DNA was electrophoresed through a 1%-agarose gel, stained, and photographed under UV light.

RESULTS

Microcapsule morphology

Human MSCs incubated in monolayer adopted a fibroblastlike morphology, as reported by others [Fig. 1(a)]. For encapsulation, about 33–35 alginate beads from 1 mL alginate/cell suspension passed from

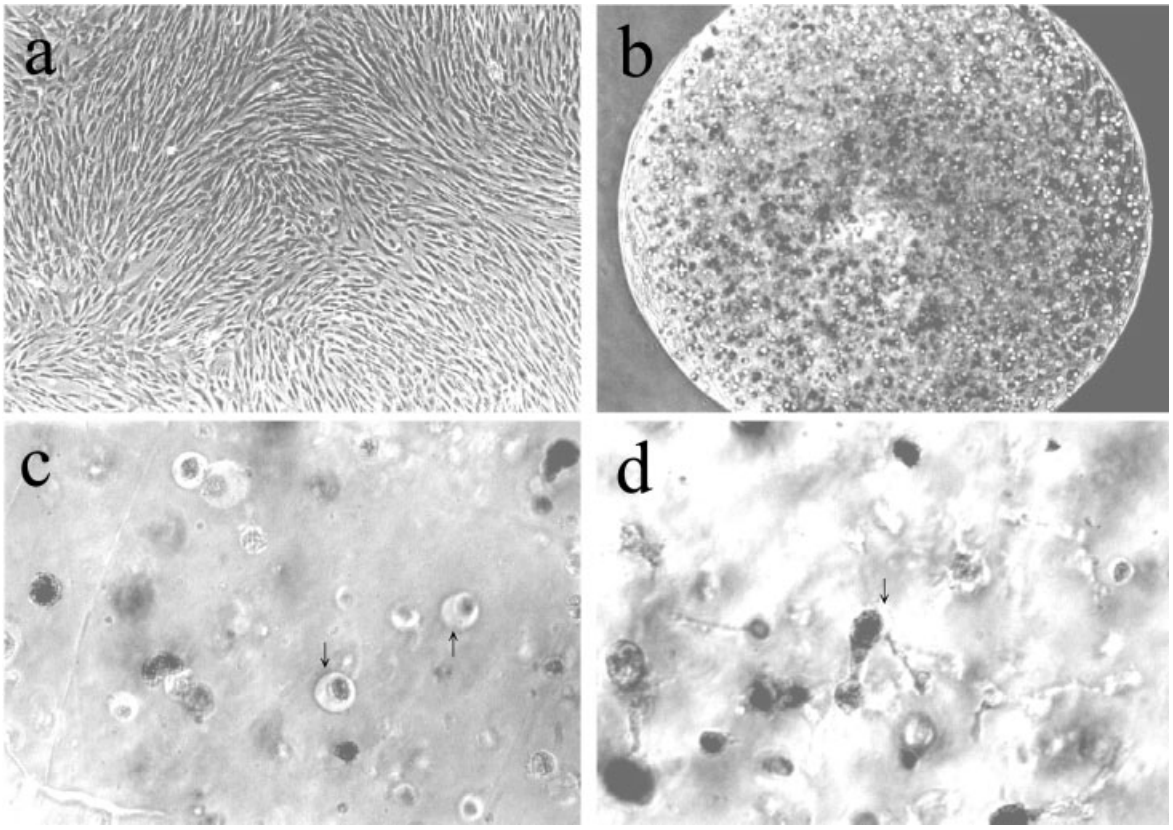

Figure 1. Phase-contrast microscopic observations of MSCs cultured in monolayer (a) and in alginate beads (b–d). (a) MSCs adopt fibroblastlike morphology in monolayer culture (original magnification ×40). (b) Day 1 of cultures: MSCs are embedded in spherical alginate beads (original magnification ×40). (c) Day 7 of cultures: encapsulated cells assume the typical rounded shape of chondrocytes, with lacunae formed around them (original magnification ×300). (d) Day 21 of cultures: during the following days, MSCs form clumps of cells (original magnification ×300).

a 16-gauge needle were gained after gelling on contact with $CaCl_2$ solution. The beads were round in shape, identical in size, with a mean diameter of 3.4 mm, and contained 6×10^4 MSCs in each bead. The MSCs were discretely scattered in the bead, and there were no differences in shape and density from the peripheral to the center of the bead throughout the 4-week experiment [Fig. 1(b)]. At 1 week after encapsulation, cells assumed a rounded morphology with lacunae around them [Fig. 1(c)]. Cells clumped to form aggregates after 2 weeks in culture [Fig. 1(d)]. With time, the aggregates enlarged to form larger nodules at 3 or 4 weeks after encapsulation.

Viability and proliferation

In alginate, the viability remained high (>90%) for the encapsulated MSCs throughout the 4-week experiment. The duration of *in vitro* culture had no effects on the viability of the cells. Proliferation was not estimated by cell number in beads but by analyzing DNA content, because the loss of cells during the resolution of alginate influenced the result. Flow cytometric analysis of cell cycle with propidium iodide indicated that encapsulation led to an increase in the number of cells in the G0G1 phase and a concomitant decrease in the number of cells in the S phase [Fig. 2(a)]. The median S-phase fractions were 27.4, 7.5, 5.0, 1.7, and 3.2% for monolayer culture versus suspension in alginate for 24, 48, 72, and 96 h, respectively (Table I). Recovered cells from alginate followed by reseeding them in monolayer restarted S-phase fractions only to 9.2, 3.9, and 5.7 after reseeding for 72 , 96, and 120 h, respectively [Fig. 2(b)] (Table I). Encapsulation in alginate provided a suspension condition for "synchronizing" MSCs in G0G1 arrest, and reseeding cells in monolayer did not extensively start the cell cycle.

TABLE I
Flow cytometric analysis of cell cycle phase after encapsulation in alginate and after reseeding in monolayer

	% G0G1	% S	% G2M
		After encapsulation	
0 hr	57.3%	27.4%	15.4%
24 hr	85.6%	7.5%	6.9%
48 hr	87.5%	5.0%	7.4%
72 hr	92.5%	1.7%	5.8%
96 hr	92.1%	3.2%	4.7%
		After reseeding	
9 hr	95.2%	0.9%	3.9%
12 hr	95.2%	0.8%	4.0%
20 hr	94.5%	1.0%	4.5%
72 hr	74.8%	9.2%	16.0%
96 hr	88.6%	3.9%	7.6%
120 hr	80.9%	5.7%	13.4%

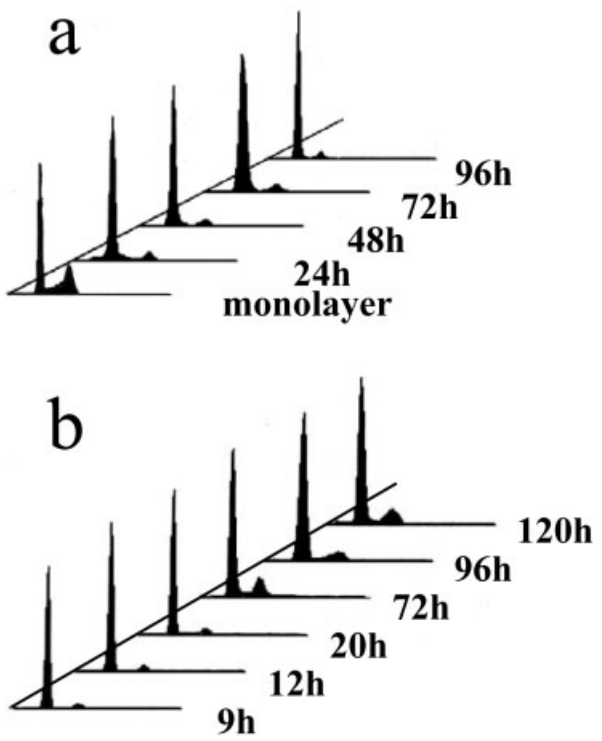

Figure 2. Flow cytometric analysis of cell cycle using propidium iodide. The MSCs were encapsulated in alginate for 24, 48, 72, and 96 h (a). Cells after release from alginate were reseeded into monolayer for 9, 12, 20, 72, 96, and 120 h (b). The cell cycle distribution of 10^5 cells for each condition was analyzed with a flow cytometer. The first and second peaks represent, respectively, the G0G1 and G2M phases of the cell cycle, and the interval between them represents the S phase. The percentages of cells in each phase for each condition are listed in Table I. (a) "Encapsulation in alginate" results in an increase in the percentage of cells in the G0G1 phase and a decrease in the percentage of cells in the S phase. (b) "Reseeding cells in monolayer" after release from alginate restarts only a small percentage of cells into the S phase.

Histology, histochemical analysis, and immunohistochemistry for collagen type II

At first, paraffin sections and cryostat sections were prepared to evaluate the specimens, but both failed. Because standard paraffin-embedding procedures caused loss of antigenicity, and cryostat sections produced sections of very poor quality, PEG embedding was used and successfully circumvented these drawbacks. Encapsulated in alginate beads, the 1-day culture showed isolated MSCs irregularly scattered in the gels [Fig. 3(a)]. They appeared as roundish cells with scant, slightly basophilic cytoplasm. The cells were completely negative for Safranin-O staining and for collagen type II both inside and around the cells [Fig.

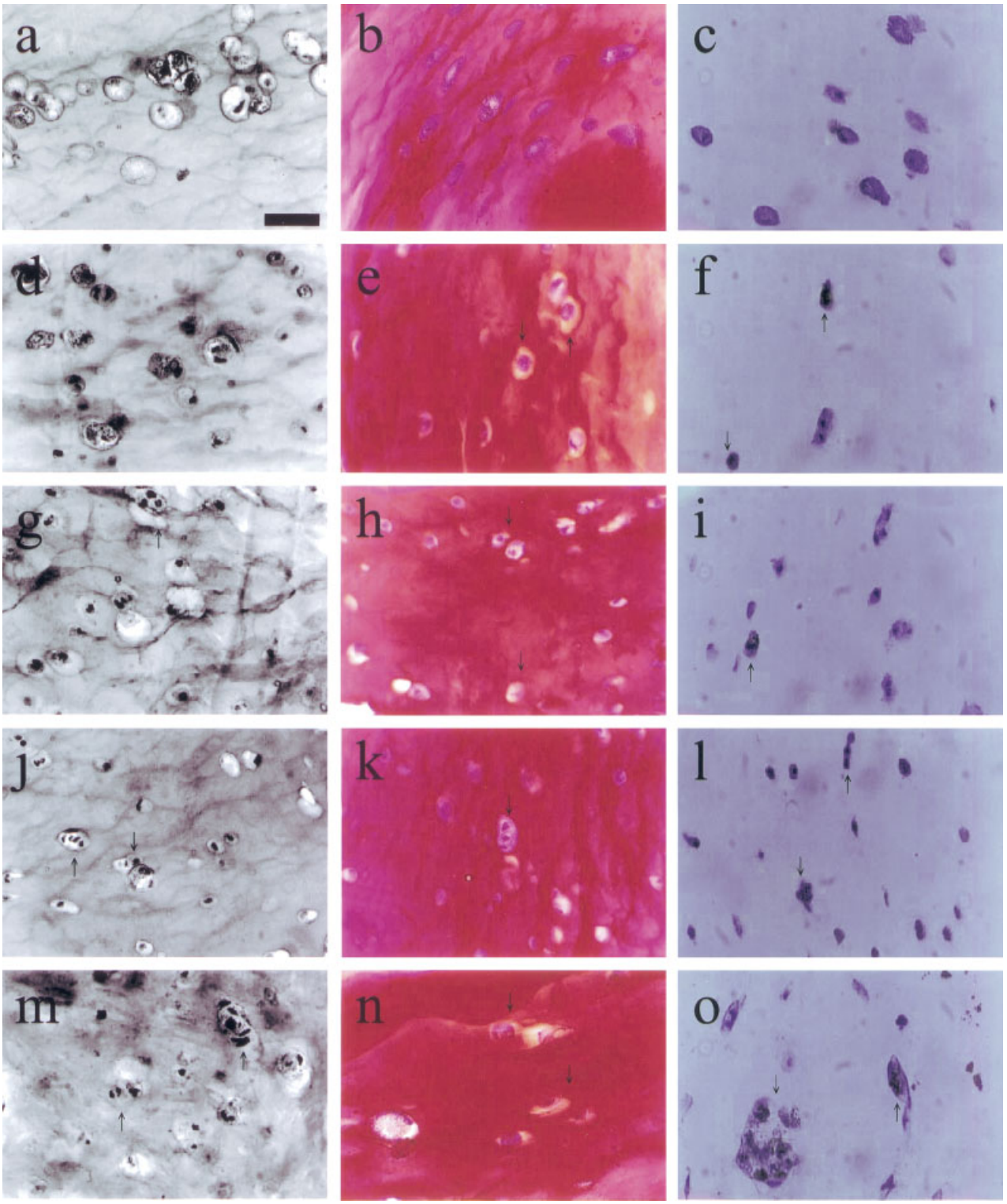

Figure 3. Polyethylene glycol (PEG) sections of MSCs in alginate for 1 day (a, b, c), 1 week (d, e, f), 2 weeks (g, h, i), 3 weeks (j, k, l) and 4 weeks (m, n, o) stained with H&E (a, d, g, j, m), Safranin-O (b, e, h, k, n), and immunohistochemistry for collagen type II (c, f, i, l, o). Lacunae (arrowheads) are visible around the cells after 1 week in alginate (d, g, j, m). Cell aggregates (arrows) are observed after 2 weeks in alginate (g, j, m). Cells and cell aggregates are surrounded by Safranin-O strongly stained matrix (arrows) after 1 week in alginate culture (e, h, k, n). Collagen type II positive matrix (arrows) is also demonstrated around the cells or cell aggregates after 1 week in alginate (f, i, l, o). Original magnification ×200. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

3(b, c)]. In the 1-week culture, the cells were present as isolated cells with basophilic lacunae around the cells [Fig. 3(d)]. Safranin-O staining was positive around the cells and the lacunae [Fig. 3(e)]. The cell was also positive for collagen type II [Fig. 3(f)]. In the 2-week culture the cells assumed a rounded morphology or grew as small aggregates in lacunae [Fig. 3(g)]. The cells in the aggregates were rounded or ellipsoidal in appearances, and Safranin-O and immunohistochemistry for collagen type II showed positive around the aggregates and the lacunae [Fig. 3(h, i)]. In the 3-week and 4-week cultures [Fig. 3(j, m)] the cells were much the same as the 2-week culture but the cells were present as large aggregates, and Safranin-O [Fig. 3(k, n)] and immunohistochemistry for collagen type II [Fig. 3(l, o)] were strongly positive around the aggregates and lacunae.

RT-PCR of COL2A1 and COL10A1

To confirm and compare the expression levels of COL2A1 and COL10A1 in each tissue sample, RT-PCR was performed using specific primers to total RNA extracted from alginate-released cells. Despite the use of equal volumes of RT products, there was some variability in the expression levels of β-ACTIN, which was used as internal control. However, specific PCR products of COL2A1 and COL10A1 were not expressed either in the monolayer culture or in the cells, 1 day in alginate culture, but were noted in all cultured cells, more than 1 week in alginate (Fig. 4). The amount of PCR product reflected the expression levels seen in immunohistochemistry. The COL2A1 and COL10A1 were amplified to maximum levels in cells cultivated in alginate for 4 weeks, which showed the highest intensity of glycosaminoglycan in Safranin-O stain and the highest expression of type II collagen in immunohistochemistry.

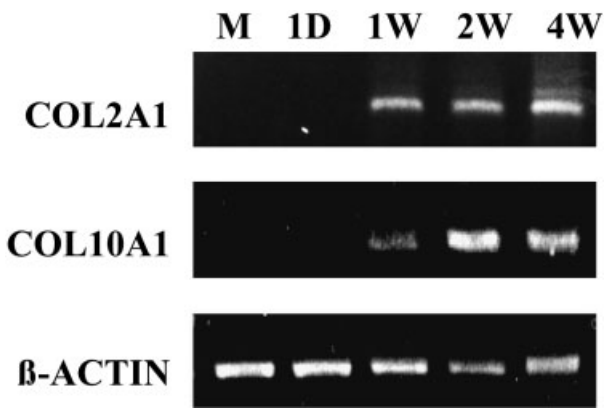

Figure 4. Analysis of the type II collagen and type X collagen mRNAs by RT-PCR. Total RNA was prepared from monolayer culture (M) and from cells released from alginate beads, which were cultivated *in vitro* for 1 day (1D), 1 week (1W), 2 weeks (2W), and 4 weeks (4W). Total RNA was analyzed by RT-PCR for expression of type II collagen (COL2A1, 399 bp), type X collagen (COL10A1, 429 bp), and β-ACTIN (515 bp).

DISCUSSION

Articular cartilage is a highly specialized tissue consisting of chondrocyte and an organic matrix comprising mainly water, proteoglycan, and type II collagen. In addition, cartilage is avascular and not innervated. Adequate, long-term repair of cartilage defects still remains an indefinable goal. Recently, many efforts have been undertaken to repair articular cartilage lesions by implantation of chondrocytes embedded and distributed in a suitable delivery substance. Collagen gels showed a significant increase in cell numbers, but the chondrocytes dedifferentiated into fibroblastlike cells after a short culture period.[11] Polymer, such as polylactic acid, is too rigid and will affect the ability to seed cells and mold the construct into defects.[29] In contrast, agarose is not sufficiently rigid or durable to use as a carrier.[30] In "semisolid" alginate beads, the chondrocytes assumed a polygonal-rounded morphology, grew as small clumps, and gradually increased proteoglycan and type II collagen synthesis.[11,20,21]

The chondrogenesis of embryonic cells[31] and the chondrocyte phenotype restoration of dedifferentiated chondrocytes[23,32] have both been developed in alginate culture. Nevertheless, the chondrogenesis of bone marrow MSCs in alginate beads is not well studied, with the exception of a rabbit model using marrow stromal cells embedded in alginate for repair of osteochondral defects.[33] However, the cells used for repair were not labeled and traced; furthermore, the empty defect and defect implanted with alginate alone were both filled with chondrocytes. Previous studies have shown that microencapsulation using calcium alginate maintains the chondrogenic phenotype of articular cartilage *in vitro*.[27] Our data prove that culture in this encapsulation system induces the chondrogenic phenotype in bone marrow MSCs, as well. To our knowledge, this is the first report that chondrogenesis of adult human MSCs has been achieved in alginate culture. Therefore, microencapsulation system using calcium alginate may provide a delivery substance and three-dimensional scaffold for tissue engineering of cartilage. Marrow MSCs offer several advantages over chondrocytes with regard to acting as a cell source for tissue engineering of cartilage: they are easily obtained, are abundant after expansion, and contain pluripotent stem cells that have the ability to differentiate along both osteogenic and chondrogenic cell line. This is clearly advantageous in the repair of osteochondral

defect where the restoration of both the subchondral bone and articular surface is necessary.

Cell death acquired during encapsulation by a temporary reaction to the toxicity of the $CaCl_2$ should be considered. Thus, the viability of cells encapsulated in alginate beads should be determined. The growth potential inherent to different cell lines is a more important determinant of survival than are the environmental factors offered by the capsule types.[34] Previous studies have shown that calcium alginate maintains high viability for articular cartilage *in vitro*.[11,20] Our data showed that more than 90% of the encapsulated MSCs survived throughout the 4-week experiment *in vitro*.

The proliferation capacity of bone marrow MSCs was inhibited in alginate culture, as shown by nearly complete G0G1 arrest noted 3 to 4 days after encapsulation. Proliferation was not extensively regained after recovery from alginate and reseeding into culture plastic dish. The proliferation of MSCs is therefore anchorage-dependent and alginate encapsulation provides a suspension condition, which can "synchronize" and arrest cells in G0G1 phase. Synchronization is a useful method for investigating mechanism involved in the cell cycle.[35] The MSCs can proliferate without differentiation to 1 billion–fold increase in number, and differentiate into different tissues under specialized conditions.[28] In addition, cells encapsulated in alginate gel can be easily recovered by the addition of a chelating agent. The MSCs-alginate system, therefore, is a practical model to investigate the molecular mechanisms involved in cell cycle and to determine the relationship between proliferation and differentiation. The cells freshly recovered from alginate by chelating agents did not retain their replication capacity after reseeding into culture plastic dish, indicative of the fact that the chondrogenic differentiation of MSCs was in progress within alginate in the addition of TGF-β1 in the medium.

On PEG sections, cell cluster at the lacunae, surrounded by Safranin-O strongly stained matrix, was found at 1 week after alginate culture. Immunohistochemistry also demonstrated strong positivity around the cells. The synthesis of the two main matrices of articular cartilage, proteoglycan and type II collagen, represents that MSCs embedded in alginate beads with the addition of TGF-β1 and dexamethasone in the medium can undergo chondrogenesis. The alginate encapsulation system, especially, can provide a delivery substance and three-dimensional scaffold for tissue engineering in cartilage. The permeability of macromolecule of alginate beads has been studied. Molecule less than 49 kDa can readily penetrate the pores of the alginate beads.[20] Human TGF-β1, a 25-kDa protein, therefore can enter the beads to regulate the proliferation and differentiation of cells in the alginate.

In vitro model systems of chondrogenesis and endochondral ossification provide a valuable tool for studying differentiation of cartilage and bone formation and matrix mineralization. In addition, to investigate the effects of biomaterials on cells, a number of laboratories have explored the use of cell cultures. However, the *in vitro* model of chondrocytes in monolayer is limited in applications, because the cells tend to lose their *in vivo* morphological phenotype of chondrocytes.[26] In this article, we show that culture of human MSCs in alginate beads results in the expression of type II collagen, one of the differentiation markers for chondrocytes. Type II collagen gene expression was clearly induced from 1 week after alginate bead inclusion and paralleled protein expression, as assessed by immunohistochemistry. These data support the alginate encapsulation system as a tool for studying the process of chondrogenesis. The cells also gave rise to the expression of type X collagen, a marker for hypertrophic cartilage undergoing endochondral ossification[36,37] and for articular chondrocytes that have become hypertrophic in osteoarthritis.[38] Additionally, the longer the cells are cultured, the stronger is the expression of type X collagen. This study provides new evidence of the usefulness of the alginate system for studying endochondral ossification. Furthermore, this model may be helpful for the analysis of molecular mechanisms underlying chondrogenesis and endochondral ossification during development and repair and could be used for testing factors such as TGF-β1,[39] which can modulate the proliferation and chondrogenic differentiation of MSCs in an *in vitro* environment resembling the *in vivo* situation.

In the current study, human MSCs isolated form bone marrow and cultured in alginate gel underwent chondrogenesis and endochondral ossification. These data indicate that bone marrow–derived MSCs can go on endochondral ossification to begin mineralization.[40] However, bovine articular chondrocytes maintained for 8 months in alginate gel continue to synthesize cartilage-specific type II collagen, and did not synthesize any type X collagen.[27] The MSCs and articular chondrocytes therefore were found to differ in terms of differentiation potential. These data indicate that MSCs can be a research object to determine the mechanisms involved in chondrogenesis and endochondral bone formation.

Several important advantages of this system over the use of other gel matrices used for entrapment are: (1) the simple and readily manipulated system for encapsulated cells, (2) a delivery substance and three-dimensional scaffold for tissue engineering in cartilage, (3) the ready dissolution of alginate beads by chelating agent such as EDTA. Bonaventure et al.[41] reported that the extraction of total RNA from cells cultured in alginate was much easier than in agarose and provided good yield of undegraded RNA. The

use of collagenase also improved the recovery of total RNA from embedded chondrocytes in alginate.[27]

In conclusion, MSCs subcultured for multiplication and then cultivated in alginate beads for more than 1 week for chondrogenesis represent an attractive alternative chondrocyte source for tissue engineering in cartilage. In particular, it should be useful when normal cartilage is not available in sufficient amount, such as in large osteochondral defects or diffuse osteoarthritic changes.

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