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ORIGINAL ARTICLES

Hyaluronic Acid Enhances Proliferation and Chondroitin Sulfate Synthesis in Cultured Chondrocytes Embedded in Collagen Gels

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The effects of hyaluronic acid (HA) on the proliferation and chondroitin sulfate (CS) synthesis of chondrocytes embedded in collagen gels were examined. Articular cartilage was isolated from the humerus, femur, and tibia of 21 10-week-old Japanese white rabbits. Chondrocytes isolated by collagenase digestion were embedded in type I collagen gels and cultured in Dulbecco's modified Eagle's medium (DMEM) with various doses of HA for 4 weeks. Histological and biochemical evaluations were performed at postculture weeks 1, 2, 3, and 4. For biochemical evaluations, isomers such as chondroitin 6-sulfate (Δdi-6S) and chondroitin 4-sulfate (Δdi-4S) synthesized by cultured chondrocytes were determined by high performance liquid chromatography (HPLC) combined with fluorometry. Morphological and histological studies demonstrated that HA-treated chondrocytes in collagen gel proliferated profusely while maintaining their phenotype. At postculture week 4, 0.1 mg/ml of HA induced an eightfold increase in cell counts compared with HA pretreatment values, or 1.5-fold more than control group. Synthesis of Δdi-6S (Δdi-6S content/cell) in groups treated with 0.01 and 0.1 mg/ml of HA significantly increased, while gel accumulation rates in groups treated with 0.1 and 1.0 mg/ml of HA scored significantly higher values than other groups. In collagen gel culture, HA enhanced the proliferation and Δdi-6S synthesis of chondrocytes while maintaining their phenotype. In clinical application, since the supply of autologous chondrocytes for transplantation is not unlimited, the HA-treated culture method may be useful for increasing the number of chondrocytes and thus improving the quality of implants. J. Cell. Physiol. 179: 142–148, 1999. © 1999 Wiley-Liss, Inc.

Numerous attempts to repair full-thickness defects in articular cartilage, including chondrocyte transplantation, have been conducted in many experimental studies (Chesterman and Smith, 1968; Bentley and Greer, 1971; Green, 1977; Mitchell and Shepard, 1976; Aston and Bentley, 1986). Although the histological appearance and functions of these repaired tissues resemble those of hyaline cartilage, these tissues deteriorate with time, downgrading to fibrous tissues to finally succumb to joint destruction.

Recently, Brittberg et al. (1994, 1996) have reported that a cartilaginous defect in the knee joint was successfully treated with transplantation of autologous chondrocytes cultured in a monolayer system. We, however, have two concerns about this culture method for the chondrocytes: (1) maintenance of chondrocyte phenotypes during prolonged culture and (2) the risk of chondrocyte leakage from a graft site after a range of motion exercise and loading on the periosteal patch. Therefore, Ochi et al. (1998) have clinically attempted to restore an osteochondral defect with transplantation of autologous cultured chondrocytes embedded in type I collagen gel. This report is based upon the fact that transplantation of chondrocyte-collagen composites to repair full-thickness defects has proved to be a promising method for cartilage repair with hyaline cartilage in experimental models (Wakitani et al., 1989, 1994). It is known that when chondrocytes are cultured in collagen gels to furnish a three-dimensional structure without altering the phenotype, the structure portrays an extracellular matrix composed of glycosaminoglycans (GAG) and type II collagen, forming an architecture that resembles hyaline cartilage (Yasui et al., 1982; Kimura et al., 1987). However, it is more difficult to proliferate chondrocytes in collagen gel than in monolayer cultures (Yasui et al., 1982; Kimura et al., 1987). Autologous chondrocytes obtained from a non-weight-bearing site are limited. It is therefore essential to establish successful transplantation by increasing the number of chondrocytes in vitro while maintaining

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their phenotype to produce GAG that would eventually improve the quality of implants.

In recent years, investigations to improve the quality of chondrocyte-collagen implants have been extensively undertaken with several growth factors. Toolan et al. (1996) have reported that incubation for 6 weeks in the presence of basic fibroblast growth factor (bFGF) and insulin induces articular cartilage repair in metabolically and mitotically activated chondrocyte-collagen implants. However, Matsusaki et al. (1998) have previously reported that human recombinant bFGF suppresses chondroitin sulfate (CS)–GAG synthesis. The effects of FGF on chondrocyte-collagen composites therefore remain controversial. Therefore, there is an essential need for safe materials, including current chemotherapeutics for innovating useful implants appropriate for chondrocyte proliferation while maintaining their phenotype besides inducing GAG production and type II collagen expression.

Hyaluronic acid (HA), which reportedly affects proliferation (Yoneda et al., 1988), migration (Hakkanson and Venge, 1985), and differentiation (Toole and Gross, 1971) of various animal cells, has been employed extensively in the treatment of osteoarthritis patients without complications (Iwata, 1993), although controversial studies have been documented on the effects of HA on GAG synthesis of chondrocytes. HA has been reported to elicit inhibitory (Wiebkin and Muir, 1973; Solursh et al., 1974, 1980) and no effects (Mason et al., 1989; Larsen et al., 1991; Shimazu et al., 1993) on cultured chondrocytes in vitro. If in a three-dimensional culture HA enhanced chondrocyte proliferation as well as syntheses of GAG and type II collagen, chondrocyte-collagen composites of much higher quality could then be innovated or developed. The present study endeavored to clarify the effect of HA in this culture system on chondrocyte proliferation and synthesis of CS-GAG, which is a major and an important component in articular cartilage.

MATERIALS AND METHODS

Materials

Dulbecco's modified Eagle's medium (DMEM) (Gibco, Grand Island, NY), fetal bovine serum (FBS), and antibiotics consisting of penicillin/streptomycin/amphotericin B (JR Scientific, Woodland, CA) were used for the cultures. Trypsin (Difco Lab, Detroit, MI), type I and II clostridial collagenases (Sigma Chemical Co., St. Louis, MO), and Cellmatrix® (0.3% acid-dissolved type I collagen derived from porcine tendon (Nitta Gelachin, Tokyo, Japan) for chondrocyte isolation were prepared before the experiment. The HA (Seikagaku Corporation, Tokyo, Japan) used in the study, which has a viscometric average molecular weight of 8×10^5 (viscosity: 11.8–19.5 dl/g), was derived from chicken combs. The highly pure (over 99%) HA elicited no cross-reactivities with other proteins and was free of endotoxins, microorganisms, other GAGs, and proteins.

Isolations of chondrocytes

Twenty-one 10-week-old Japanese white rabbits with a mean body weight of 1.9 kg (range: 1.8–2.0 kg) were used in this study. Articular cartilage slices, gathered from the knee, hip, shoulder, and elbow joints of animals used in this study, were detached from adherent connective tissues before being cut into smaller pieces. Chondrocytes were then isolated by enzymatic digestion as previously reported (Yasui et al., 1982). Briefly, cartilage specimens were minced and washed three times in sterile 0.9% sodium chloride before isolating the chondrocytes with 0.25% trypsin in sterile saline for 30 min followed by 0.25% collagenase in DMEM supplemented with 10% heat-inactivated FBS and 1% penicillin (100 IU/ml)/streptomycin (100 μg/ml)/amphotericin B (0.25 μg/ml) for 4–8 h at 37°C in a culture bottle.

Embedding chondrocytes in collagen gels

The isolated cells were collected by centrifugation (1,500*g*, 37°C, 5 min) after removal from the culture bottle and washed three times with the culture medium prior to embedding them in Cellmatrix® containing the culture medium (culture medium: Cellmatrix® = 1:8). The final cell density was adjusted to 2×10^6 cells/ml in the collagen cell medium mixture before placing 125 μl of this mixture in a 20 mm diameter culture dish (Falcon, Becton Dickinson, Oxnard, CA). The cell–collagen gel composites were further incubated at 37°C for 30 min. The gel thus formed was layered with 2 ml of culture medium. Cell cultures were incubated in 5% carbon dioxide/95% air at 37°C and replaced with fresh DMEM containing L-ascorbic acid (50 μg/ml) twice a week.

Concentration of hyaluronic acid

In this study, HA was administered at three different doses. In the control group, the gel was incubated with fresh culture medium (HA: 0 mg/ml) alone, whereas the remaining three experimental groups were treated with HA such that their final HA concentrations were 0.01 (0.1%), 0.1 (1%) and 1 mg/ml (10%), respectively. HA was added during initial seeding and replacement of the medium.

Number of chondrocytes

At postculture weeks 1, 2, 3, and 4, chondrocytes were collected by incubation with a solution containing 0.25% trypsin and 0.25% collagenase in DMEM at 37°C for 30 min. The cell suspension was collected, and the number of viable cells was counted using a hemocytometer with the trypan blue dye exclusion test (Grande et al., 1989). Cells clearly differed from the morphology of rounded chondrocytes, and dishes with less than 90% viable cells were excluded from this study. We examined six dishes twice, yielding a total of 12 dishes per group (six dishes for cell count and quantitation of CS-GAG and six for histological examinations). Ten chondrocyte–collagen gel samples were randomized before culture. The growth rates of HA-treated chondrocytes were determined at postculture weeks 0, 1, 2, 3, and 4.

Histological examination

Phase photomicrography of the cultured chondrocyte-collagen composites was performed at various incubation times. Immunohistochemical evaluations of the specimens were conducted at postculture weeks 1, 2, 3, and 4. Immunohistochemical stains were performed with S-100 antibody (specific for chondrocytes and Schwann cells) (Dako Immunoglobulin, Glostrup,

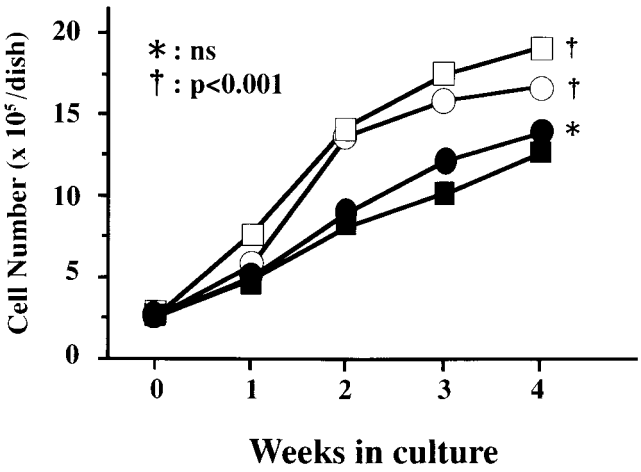

Fig. 1. Growth curves of cells in chondrocyte-collagen implants at 0 (—■—), 0.01 (—●—), 0.1 (—□—), and 1.0 (—○—) mg/ml of hyaluronic acid (HA). Each symbol represents the mean (±SD) of six samples.

Denmark) and anti-type II collagen antibody (Fuji Pharm. Lab., Toyama, Japan) to detect expression of the chondrocyte phenotype and type II collagen production, respectively.

Analysis of chondroitin sulfate

CS contents were quantified according to a previously described method (Shinmei et al., 1992). Briefly, each gel sample was washed three times with phosphate buffered saline (PBS) before digesting with 0.25% collagenase in DMEM for 45 min at 37°C. Cells were removed from the treated gel sample and harvested by centrifugation at 1,500*g* for 15 min at room temperature. The supernants were stored in aliquots at −20°C before use. Supernant samples were diluted tenfold with distilled water before digestion with chondroitinase ABC (CHase ABC) (Seikagaku Corporation, Tokyo, Japan) and hyaluronidase derived from *Streptococcus dysgalactonase* (HAase SD) (Seikagaku corporation, Tokyo, Japan). The mixture was ultrafiltered after digestion with HAase SD, and the filtrate was analyzed by high performance liquid chromatography (HPLC). HPLC analysis of the unsaturated disaccharides, derived from isomers of chondroitin 4-sulfate (Δdi-4S) and chondroitin 6-sulfate (Δdi-6S) in the filtrate, was performed according to the method of Toyoda et al. (1989).

Statistical analysis

Results were expressed as the mean plus or minus standard deviation (SD), and statistical comparisons of the means were performed using multivariate analysis of variance (ANOVA). Differences where $P < 0.05$ were considered significant.

RESULTS

Effect of HA on chondrocyte counts in cultures

The chondrocyte counts on initiating culture were comparable in all samples ($2.5 \pm 0.12 \times 10^5$ per dish). Cell proliferation with various doses of HA (Fig. 1) revealed that the number of chondrocytes treated with

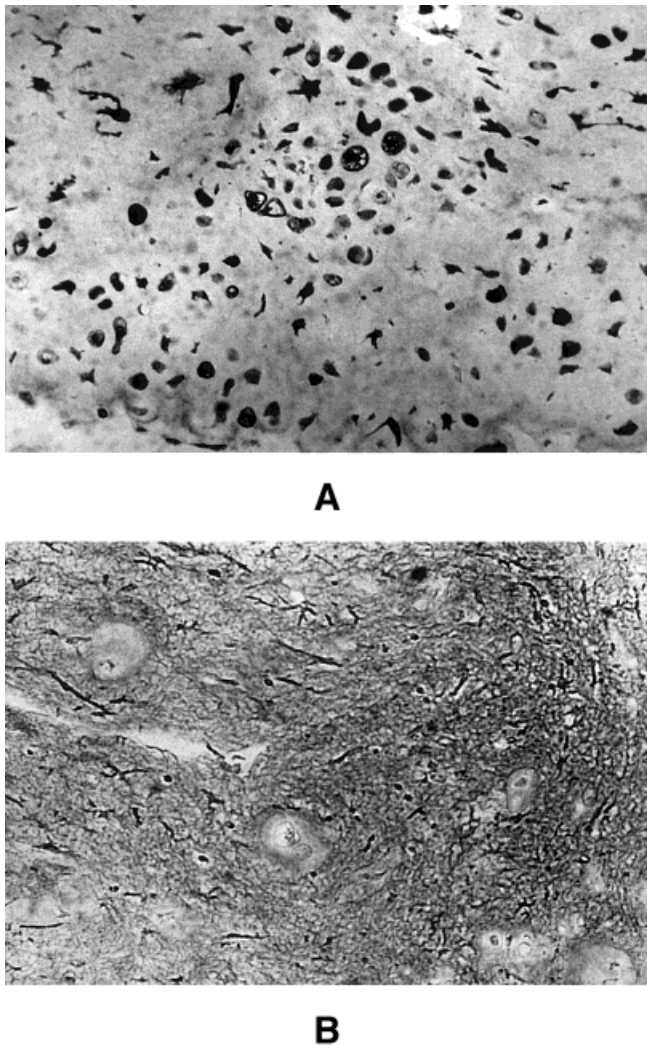

Fig. 2. Photomicrographs of chondrocyte-collagen composites showing immunohistochemistrical stains of 0.1 mg/ml hyaluronic acid (HA) at postculture week 4. **A:** With S-100 antibody. ×200. **B:** With anti-type II collagen antibody. ×200.

HA at 0.1 and 1.0 mg/ml increased gradually to reach the stationary phase at postculture week 3. Chondrocytes treated with 0.1 mg/ml HA registered the highest count ($19.2 \pm 1.01 \times 10^5$) among all groups at the designated culture periods to finally establish a population eightfold higher than the preculture value, or attaining a count 1.5-fold more than that of controls ($12.7 \pm 1.41 \times 10^5$). Verification with ANOVA revealed that HA induced chondrocyte proliferation ($P < 0.001$) in a dose-dependent manner.

Morphology and histological evaluations

Throughout the culture periods, phase photomicrography consistently presented cultured chondrocytes to perpetuate as rounded structures without altering their morphology. In the group treated with 0.1 mg/ml HA, the gel was filled with rounded cells that nearly attained confluence at postculture week 3. Immunohistochemical staining with S-100 protein antibodies re-

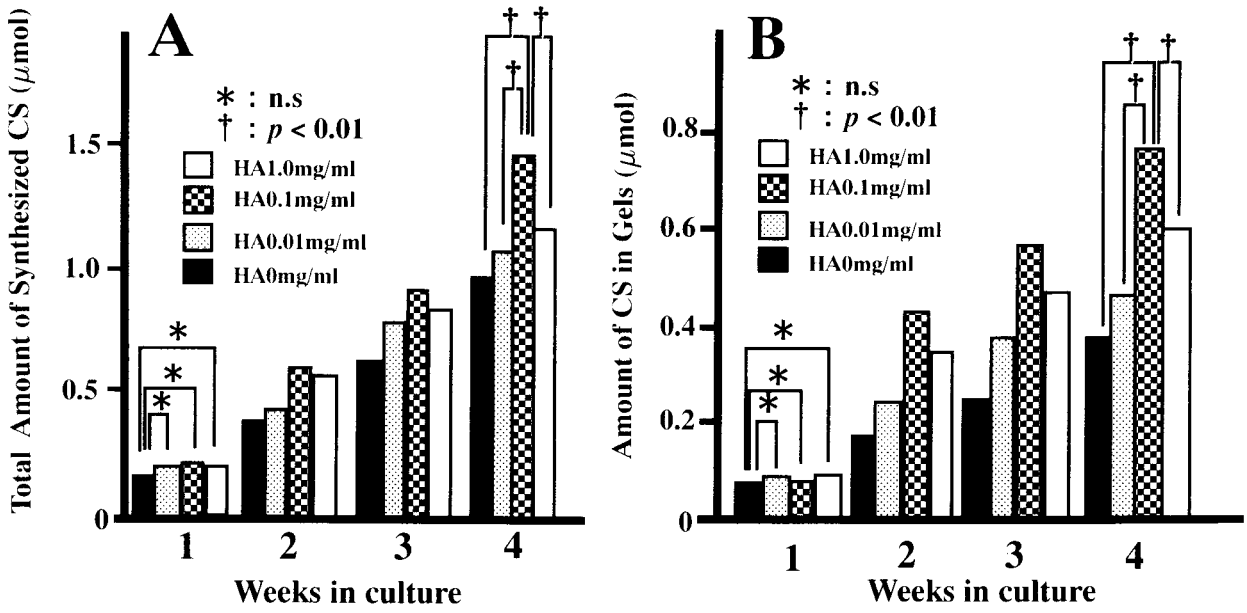

Fig. 3. Effects of HA on chondroitin sulfate (CS) GAG synthesis at various doses of hyaluronic acid (HA) with respect to the culture period. Each column with a bar represents the mean ± SD of six samples. Total amounts of CS (Δdi-6S + Δdi-4S) synthesized by cultured chondrocytes in gels + culture medium (**A**) and collagen gels (**B**) at every postculture week. Statistical significance where $P < 0.01$ (†) and insignificance (n.s.: *) were verified by ANOVA analysis.

vealed that cells grown in collagen gels in all groups appeared round and responded positively to anti-S-100 immunoreaction every postculture week without any change in phenotype (Fig. 2A). Immunohistochemical staining with anti-type II collagen antibodies also demonstrated that type II collagen was produced (Fig. 2B).

Amount of chondroitin sulfate

There was a time-dependent increase in CS (Δdi-6S + Δdi-4S) synthesis (Fig. 3A). Among all groups, the 0.1 mg/ml HA-treated group had the highest ($P < 0.01$) CS content (1.486 ± 0.044 μmol) at postculture week 4. The mean total CS contents in collagen gels increased rapidly at postculture week 2 and continued to increased thereafter (Fig. 3B). In postculture week 4, similar to data on total CS synthesized, the 0.1 mg/ml HA-treated group scored the highest ($P < 0.01$) mean total CS (0.820 ± 0.026 μmol) compared with the other groups.

Effects of HA on CS, Δdi-6S, or Δdi-4S synthesis

No HA-dependent differences were noted in CS, Δdi-6S, or Δdi-4S synthesis at postculture week 1. At postculture week 4, the total amount of CS synthesized registered 98, 99, and 92%, respectively, in the groups treated with HA at 0.01, 0.1, and 1.0 mg/ml compared to the control group (100%). Although insignificant with regard to CS contents at postculture week 4, Δdi-6S synthesis in groups treated with HA at 0.1 and 0.01 mg/ml indicated significant increases ($P < 0.01$) (Fig. 4A), with the former and latter registering, respectively, 110% and 114% against the controls. Δdi-4S synthesis, however, was suppressed by HA dose-dependently. Compared with the control group (100%), mean Δdi-4S synthesis registered 92% ($P < 0.05$), 78% ($P < 0.01$), and 76% ($P < 0.01$) in groups treated with 0.01, 0.1, and 1.0 mg/ml HA, respectively (Fig. 4B).

Effects of HA on the Δdi-6S/Δdi-4S ratio

The Δdi-6S/Δdi-4S ratios of CS in collagen gel (Fig. 5A) and both collagen gel and culture medium (Fig. 5B) showed significant increases ($P < 0.01$ vs. control) in groups treated with 0.1 mg/ml HA compared with other doses. However, differences in the Δdi-6S/Δdi-4S ratio of culture medium between any two of the HA-treated groups were insignificant.

DISCUSSION

In this study, HA enhanced both proliferation and CS-GAG content, especially Δdi-6S isomer synthesis, in long-term cultured chondrocytes embedded in collagen gels. Our report further demonstrated two new aspects of HA effects on chondrocytes using a three-dimensional culture system: (1) qualitative physiological effects of exogenous HA on articular chondrocytes embedded in type I collagen gel and (2) quantitative determination of chondrocyte-derived CS-GAG contents with HPLC fluorometry.

Previous reports on the physiological effects of HA on chondrocytes have been confined to the use of monolayer culture systems. In the culture system we employed, chondrocytes maintain their phenotype while perpetuating proteoglycan to induce type II collagen production and eventuating a structure that much resembles hyaline cartilage instead of dedifferentiating to fibroblast-like chondrocytes in long-term cultures (Kimura et al., 1987; Yasui et al., 1982). This three-dimensional culture system renders studies on the HA effects on chondrocytes possible compared with other systems. Moreover, these chondrocyte–collagen gel

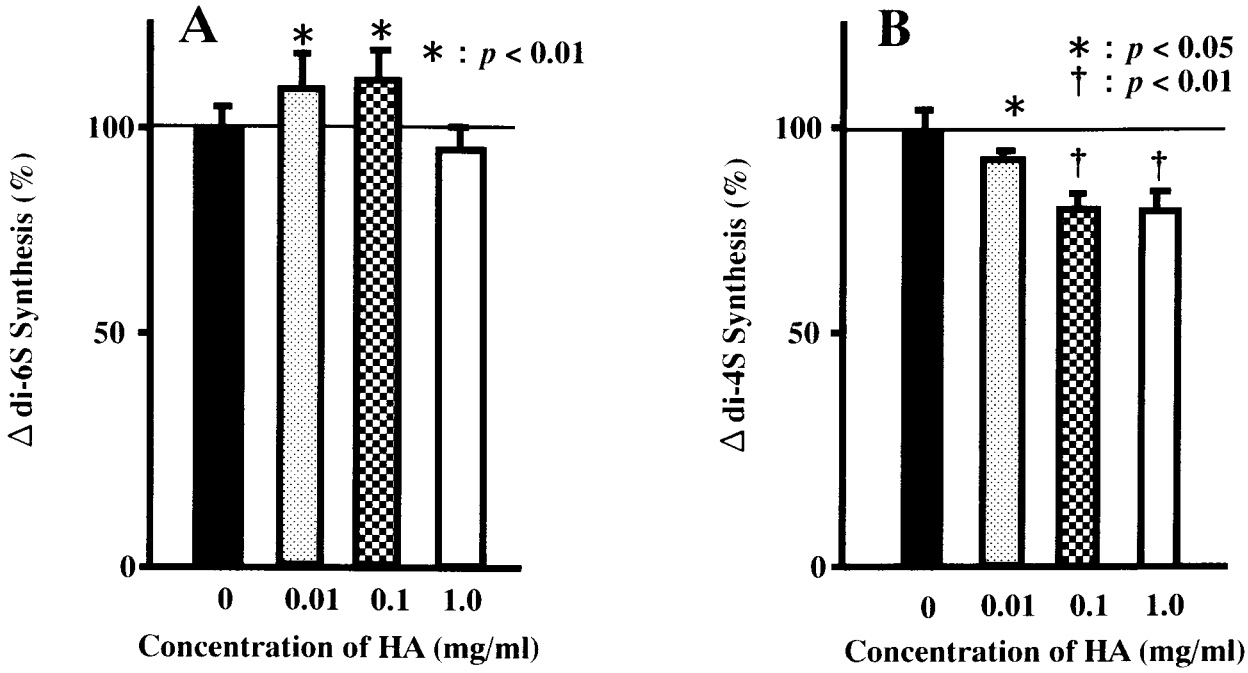

Fig. 4. Chondroitin sulfate (CS) GAG synthesis (defined as the amount of synthesized CS per chondrocyte) at various doses of hyaluronic acid (HA) at postculture week 4. CS synthesis was expressed as a percentage of the control group. Each column represents the mean ± 1 SD of six samples. **A** and **B** represent Δdi-6S and Δdi-4S syntheses, respectively. Significant differences of $P < 0.01$ (†) and $P < 0.05$ (*) were evaluated by ANOVA analysis.

composites can be transplanted for treatment of articular cartilage defects (Ochi et al., 1998). Therefore, it is important to examine the HA effects on chondrocytes in the present culture system.

In the second aspect noted above, this culture system afforded a determination method for analyzing CS-GAG synthesized by cultured chondrocytes. In previous reports (Solursh et al., 1974, 1980; Wiebkin and Muir, 1977; Larsen et al., 1991; Shimazu et al., 1993), GAG synthesis (indexed by sulfated GAG) had been measured by incorporating ^{35}S-sulfate into proteoglycan. Although this method can measure all sulfated GAG produced by cultured chondrocytes, it cannot quantitatively discriminate the isomer contents of synthesized CS-GAG. Due to the fact that a recent study (Bayliss et al., 1995) demonstrated that almost all GAG in articular cartilage exists in the form of CS-GAG, we employed an HPLC method for determinating isomers of CS-GAG in this study. This method, previously used in monitoring degradated articular cartilage in joint fluid (Shinmei et al., 1992), was able to measure isomers of CS-GAG content prevailing in the articular cartilage.

Yoneda et al. (1988) have reported that modulation of the extracellular matrix by HA may be a causal event of cell proliferation in mouse dermal fibroblasts. Our results have shown that HA at relatively high doses (≥0.1 mg/ml) might be effective in promoting proliferation of chondrocytes while maintaining the phenotype in chondrocyte-collagen composites, although the proliferation mechanism(s) is unclear. Immunostaining with anti-S-100 antibodies (Wolff et al., 1992) and anti-type II collagen antibodies (O'Driscoll et al., 1986) is useful for detection of proliferating cell types. In this study, as cells contained in the chondrocyte-collagen composites were S-100–positive and exhibited a rounded appearance, a chondrocyte phenotype was thus perpetuated. In addition, perpetuation of the positive anti-type II collagen antibody in gel induced type II collagen production while maintaining the phenotype. Since the proliferating cells stained positive with anti-S-100 protein and portrayed positive anti-type II collagen antibody immunohistostaining, their phenotype was definitely maintained in this study.

By measuring both Δdi-6S and Δdi-4S contents, our study demonstrated that enhancement of Δdi-6S synthesis was achieved by HA treatment at 0.01–0.1 mg/ml, although statistical significance on total CS synthesis (total CS per cell) at various concentrations of HA was not achieved (data not shown) at postculture week 4. These findings support previous reports (Wiebkin and Muir, 1973; Solursh et al., 1974, 1980; Mason et al., 1989; Larsen et al., 1991; Shimazu et al., 1993). Recent reports have suggested that more than 90% of the CS isomers in human adult articular cartilage exist as Δdi-6S (Bayliss et al. 1995), while ligaments and synovial capsules contain predominantly Δdi-4S, indicating that Δdi-4S originates from the fibrous tissues (Toyoda et al., 1989). HA might suppress Δdi-4S synthesis in collagen gel–embedding culture systems and instead stimulate Δdi-6S synthesis, the dominant CS isomer in articular chondrocytes. These results suggest

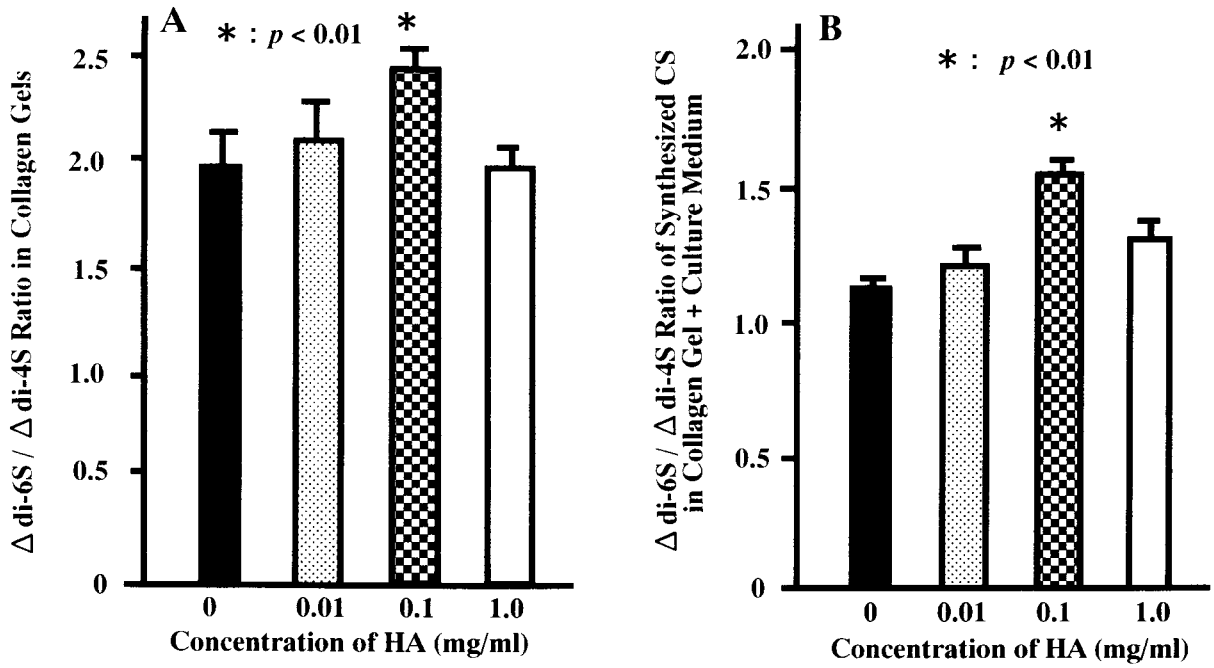

Fig. 5. Δdi-6S/Δdi-4S ratios at various doses of hyaluronic acid (HA) at postculture week 4. Each column with a bar represents mean ± SD of six samples. Δdi-6S/Δdi-4S ratios of synthesized CS were derived in collagen gels (**A**) and collagen gels + culture medium (**B**). Significant difference of $P < 0.01$ (*) was verified according to ANOVA analysis.

that HA might not stimulate syntheses of all GAG apart from the chondrocyte-dominant GAG (Δdi-6S).

Bayliss et al. (1995) have further demonstrated that healthy mature cartilage displays a higher Δdi-6S/Δdi-4S ratio than immature cartilage. In addition, healthy articular cartilage furnishes Δdi-6S–rich isomers compared with osteoarthritis cartilage (Hardingham and Fonsang, 1992). This was manifested by significantly higher Δdi-6S/Δdi-4S ratios in collagen gels than in culture media. Furthermore, addition of 0.1 mg/ml HA enhanced the Δdi-6S content to improve the quality of an implant, making it comparable to that of healthy cartilage. Therefore, HA may produce Δdi-6S–rich implants for articular cartilage defects.

There is an optimum concentration of HA in CS-GAG synthesis of collagen gel cultures. In our study, an HA concentration range of 0.01–0.1 mg/ml enhanced Δdi-6S synthesis in the culture system used. Since HA (≥0.1 mg/ml) induced chondrocyte proliferation and inhibited displacement of synthesized CS in the culture medium, the optimum concentration for HA effects in our culture system may therefore be 0.1 mg/ml.

The results of this study demonstrated that type I collagen gels served well as a delivery system that supports chondrocyte phenotypic expression in vitro and incorporates CS synthesized by these cells. Moreover, addition of HA into the culture medium enhanced the metabolic activities of chondrocytes, facilitating formation of composites with much enriched CS-GAG, especially the predominant Δdi-6S GAG in articular cartilage. Our findings suggest that transplantation of these implants may provide more promising beneficial repairs to osteochondral defects. Attempts to perform such implants in animals and humans are under study in our laboratories.

LITERATURE CITED

Aston JE, Bentley G. 1986. Repair of articular surfaces by allografts of articular and growth-plate cartilage. J Bone Joint Surg [Br] 69:29–35.

Bayliss MT, Davidson C, Woodhouse SM, Osborne DJ. 1995. Chonrdoitin sulphation in human joint tissues varies with age, zone and topography. Acta Orthop Scand 66 (Suppl 266):22–25.

Bentley G, Greer RB III. 1971. Homotransplantation of isolated epiphyseal and articular cartilage chondrocytes into joint surfaces of rabbits. Nature 230:385–388.

Brittberg M, Lindahl A, Nilsson A, Ohlsson A, Isaksson O, Peterson L. 1994. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N Engl J Med 331:889–895.

Brittberg M, Nilsson A, Lindahl A, Ohlssen A, Peterson L. 1996. Rabbit articular cartilage defects treated with autologous cultured chondrocytes. Clin Orthop 326:1270–1283.

Chesterman PJ, Smith AU. 1968. Homotransplantation of articular cartilage and isolated chondrocytes: an experimental study in rabbits. J Bone Joint Surg [Br] 50:184–197.

Grande DA, Pitman MI, Peterson L, Menshe DS, Klein M. 1989. The repair of experimentally produced defects in rabbit articular cartilage by autologous chondrocyte transplantation. J Orthop Res 1:208–218.

Green WT Jr. 1977. Articular cartilage repair: behavior of rabbit chondrocytes during tissue culture and subsequent allografting. Clin Orthop 124:237–250.

Hardingham TE, Fonsang AJ. 1992. Proteoglycans: many forms, many functions. FASEB J 6:861–870.

Hakkanson L, Venge P. 1985. The combined action of hyaluronic acid and fibronectin stimulates neutrophil migration. J Immunol p 2735–2739.

Iwata H. 1993. Phamacologic and clinical aspects of intraarticular injection of hyaluronate. Clin Orthop 289:285–291.

Kimura T, Yasui N, Ohsawa S, Ono K. 1987. Chondrocytes embedded in collagen gels maintain cartilage phenotype during long-term cultures. Clin Orthop 186:231–239.

Larsen NE, Lombard KM, Parent EG, Balazs EA. 1991. Effect of

hylan on cartilage and chondrocyte cultures. J Orthop Res 10:23–32.
Mason RM, Crossman MV, Sweeney C. 1989. Hyaluronan and hyaluronan-binding proteins in cartilage tissue. In: Laurent TC, editor. The biology of hyaluronan. New York: John Wiley & Sons. p 107–121.
Matsusaki M, Ochi M, Uchio Y, Shu N, Kurioka H, Kawasaki K, Adachi N. 1998. Effects of basic fibroblast growth factor on proliferation and phenotype expression of chondrocytes embedded in collagen gel. Gen Pharmacol 31:759–764.
Mitchell N, Shepard N. 1976. The resurfacing of adult rabbit articular cartilage by multiple perforations through the subchondral bone. J Bone Joint Surg [Am] 58:230–233.
Ochi M, Uchio Y, Matsusaki M, Wakitani S, Sumen Y. 1998. Cartilage repair—a new surgical procedure of cultured chondrocyte transplantation. In: Chan KM, Fu F, editors. Controversies in orthopaedic sports medicine. Philadelphia: Lippincott-Ravin. p 549–563.
O'Driscoll SW, Keeley FW, Salter RB. 1986. The chondrogenic potential of free autogenous periosteal grafts for biological resurfacing of major full-thickness defects in joint surfaces under the influence of continuos passive motion. An experimental investigation in the rabbit. J Bone Joint Surg [Am] 68:1017–1035.
Shimazu A, Jikko A, Iwamoto M, Koike T, Yan W, Okada Y, Shinmei M, Nakamura S, Kato Y. 1993. Effects of hyaluronic acid on the release of proteoglycan from the cell matrix in rabbit chondrocyte cultures in the presence and absence of cytokines. Arthritis Rheum 36:247–253.
Shinmei M, Miyauchi S, Machida A, Miyazaki K. 1992. Quantitation of chondroitin 4-sulfate and chondroitin 6-sulfate in pathologic joint fluid. Arthritis Rheum 35:1304–1308.
Solursh M, Vaerewyck SA, Reiter S. 1974. Depression by hyaluronic acid of glycosaminoglycan synthesis by cultured chick embryo chondrocytes. Dev Biol 41:233–244.
Solursh M, Hardingham TE, Hascall VC, Kimura JH. 1980. Separated effects of exogenous hyaluronic acid on proteoglycan synthesis and deposition in pericellular matrix by cultured chick embryo limb chondrocytes. Dev Biol 75:121–129.
Toolan BC, Frenkel SR, Pachence JM, Yalowitz L, Alexander H. 1996. Effect of growth-factor-enhanced culture on a chondrocyte-collagen implant for cartilage repair. J Biomed Mater Res 31:273–280.
Toole BP, Gross J. 1971. The extracellular matrix of the regenerating newt limb: synthesis and removal of hyaluronate prior to defferentiation. Dev Biol 25:57–77.
Toyoda H, Shinomiya K, Yamanashi S, Koshiishi I, Immanari T. 1989. Microdetermination of unsaturated disaccharides produced from chondroitin sulfates in rabbit plasma by high performance liquid chromatography with flurometric detection. Anal Sci 4:381–384.
Wakitani S, Kimura T, Hirooka A, Ochi T, Yoneda M, Yasui N, Owaki K, Ono K. 1989. Repair of rabbit articular surfaces with allograft chondrocytes embedded in collagen gel. J Bone Joint Surg [Br] 71:74–80.
Wakitani S, Ono K, Goldberg VM, Caplan AI. 1994. Repair of large cartilage defects in weight-bearing and partial weight-bearing articular surfaces with allograft articular chondrocytes embedded in collagen gel. Trans Orthop Res Soc 19:238.
Wiebkin OW, Muir H. 1973. The inhibition of sulphate incorporation in isolated adult chondrocytes by hyaluronic acid. FEBS Lett 37: 42–46.
Wiebkin OW, Muir H. 1977. Synthesis of cartilage-specific proteoglycan by suspension cultures of adult chondrocytes. Biochem J 164: 269–272.
Wolff DA, Stevenson S, Goldberg VM. 1992. S-100 protein immunostaining identifies cells expression a chondrocytic phenotype during articular cartilage repair. J Orthop Res 10:49–57.
Yasui N, Osawa S, Ochi T, Nakashima H, Ono K. 1982. Primary culture of chondrocytes embedded in collagen gels. Exp Cell Biol 50:92–100.
Yoneda M, Shimizu S, Nishi Y, Yamaguchi M, Suzuki S, Kimata K. 1988. Hyaluronic acid–dependent changes in the extracellular matrix of mouse dermal fibroblasts that is conductive to cell proliferation. J Cell Sci 90:275–286.