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Full Length Research Paper

Potential of glucan HBP-A in chondrocytes-alginate hydrogel constructs

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To explore the biological characteristics of an injectable chondrocytes-alginate hydrogel, constructs in the presence of glucan HBP-A as well as its role in cartilage tissue engineering, chondrocytes were isolated from rabbit knee cartilage and verified by immunocytochemistry for type II collagen. The constructs were embedded with HBP-A and then injected to nude mice subcutaneously. Six weeks after transplantaiton, the specimens were collected for Safranin O staining and transmission electron microscopy (TEM). The mRNA expressions of disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTs-5), aggrecan and type II collegan in constructs were determined by real time PCR. Newly generated cartilaginous tissue was found after transplantation, and the collagen was obvious in the HBP-A group. In accordance with morphological observations, the mRNA expression of type II collagen was also increased significantly, accompanied by decreased expression of ADAMTs-5 ($P<0.05$) in the HBP-A group compared with the control group.

Key words: Glucan, chondrocyte, cartilage, hydrogel constructs, type II collagen.

INTRODUCTION

Cartilage repair is a major challenge faced by clinicians and scientists in the field of orthopedics, where numerous efforts have been made to “re-establish a structurally and functionally competent repair tissue” (Hunziker, 2002). In adult articular cartilage, chondrocytes account for less than 5% of total tissue volume, but are responsible for the synthesis, assembly, regulation, and maintenance of extracellular matrix (ECM). Chondrocytes can maintain normal cartilage homeostasis through the equilibrium between anabolic and catabolic activities. Aggrecan, large aggregating proteoglycan or chondroitin sulfate proteoglycan is a proteoglycan, or a protein modified with carbohydrates. Aggrecan forms a major structural component of cartilage, particularly articular cartilage. The weight-bearing capacity is dependent on the integrity and density of aggrecan and degradation and loss of aggrecan are the features of osteoarthritis (OA).

Tissue engineering strategies utilize combination of cells, biodegradable scaffolds, and bioactive molecules, representing one of the most promising approaches for restoration of damaged cartilage. The application of growth factors or some kinds of polysaccharides have received considerable attention due to their ability to induce and facilitate the matrix synthesis in and repair of cartilage. Growth factors, such as insulin-like growth factor-1 (IGF-1) and osteogenic protein-1 (OP-1), have been identified in adult articular cartilage (Schneiderman et al., 1995; Chubinskaya et al., 2000) and shown to significantly stimulate the anabolic activity of chondrocytes (Hui et al., 2003). It has been shown that chondroitin sulphate, a major functional group of polysaccharides in glycosaminoglycan (GAG), can interfere with the structural changes in joints and be used in the management of OA (Monfort et al., 2008). Therefore, some kinds of polysaccharide exert chondroprotective effect, implying its potential in cartilage tissue engineering and modifications to scaffold.

There are many kinds of natural polysaccharides. Our previous study showed that the kidney-reinforcing and liver-softening Chinese herbal compound could inhibit the

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secretion of matrix metalloproteases (MMPs)-3. Animal experiments also demonstrated that this compound could exert anti-arthralgia effects in OA (Feng et al., 2004). A main component of this compound is Oyster (Hebang) which is abundant in polysaccharide. Based on these findings, we attempted to explore the role of glucan HBP-A, a unique extract from Oyster (Hebang), in the tissue engineering. HBP-A is a kind of α-glucan composed of 1α→4 glucose residue with the molecular weight of 1.7×10^6 Dalton and the specific optical rotation $[\alpha]_D$ of +243.4° (c 0.5, H_2O). In this study, an injectable chondrocytes-alginate hydrogel composed of glucan HBP-A was prepared and its effects on cell metabolism and production of ECM were investigated aiming to explore its potential in cartilage tissue engineering.

MATERIALS AND METHODS

Isolation and culture of chondrocytes

The cartilages were obtained from the femoral condyles of 14-month-old female rabbits in the surgery. Ethics approval was obtained from the Animal Care and Use Committee at the Shanghai University of Traditional Chinese Medicine before study. The cartilages were washed in calcium and magnesium free phosphate buffered saline (PBS) and cut into pieces. Chondrocytes were released from the articular cartilage after digestion with 0.25% trypsin (Sigma, St. Louis, MO) for 5 min and subsequently for 3 h with 0.2% collagenase (Sigma) at 37°C in high-glucose Dulbecco's Modified Eagle Medium (DMEM; Life Technologies, Rockville, MD, USA) containing 100 U/ml penicillin, 100 g/ml streptomycin and 0.25 g/ml amphotericin B (Life Technologies). Chondrocytes were collected by centrifugation, washed twice, re-suspended in DMEM supplemented with 10% fetal bovine serum (FBS; Life Technologies), 25 mg/ml -ascorbic acid (Sigma) and antibiotics, and finally maintained in 100 mm dish at 37°C in a humidified air with 5% CO_2.

Immunocytochemistry of type II collagen

The chondrocyte phenotype was determined *in vitro*. Goat anti-rabbit type II collagen antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were used at 1 mg/ml. Peroxidase-conjugated goat anti-goat was used at 1:50. Endogenous peroxydase was inactivated by incubation in 0.3% H_2O_2. Non-specific staining was eliminated by treatment with 10% serum in PBS with 1% bovine serum albumin (Sigma, Saint Quentin Fallavier, France). Sections were then incubated with primary antibodies for 1 h at room temperature, or overnight at 4°C and then with horseradish peroxydase-labeled secondary antibody for 1 h. Development was performed with 3-3' diaminobenzidine (DAB) solution (Dako Corporation, CA, USA) followed by counter-staining with hematoxylin-eosin or nuclear red-eosin, dehydration, and mounting. The primary antibody was replaced with PBS serving as controls. Sections were observed under microscope (Leica Micro-Systems, Reil-Malmaison, France).

Preparation of chondrocytes-alginate hydrogel and transplantation

According to the study of Wu et al. (2004), HBP-A at 0.3 mg/ml was use in the following experiments. After 7 days of culture, chondrocytes were harvested and re-suspended in sterile 1.2% alginate in 0.15 M NaCl at a density of 4×10^6 cells /ml. The cell suspension was then dropped into 40 mM $CaCl_2$ solution, and allowed to polymerize for 10 min. According to the results of our pilot experiment, the volume ratio of alginate solution to $CaCl_2$ solution of 1:3 was optimal for the delivery. Animals were randomly divided into three of the following groups: 1) constructs with HBP-A, 2) constructs without HBP-A and 3) alginate control group. Chondrocytes-alginate hydrogel with or without 0.3 mg/ml HBP-A were immediately injected into 6-week-old BALB/c nude mice subcutaneously. Injection of alginate was also performed serving as blank control. Injection was carried out at three distinct sites (back neck and bilateral back hips). Six weeks after injection, mice were sacrificed and all injected constructs were collected. The transplants were obtained under microscope, weighed, and analyzed. All animal procedures were conducted under the approval of the Shanghai University of T.C.M Animal Care and Use Committee.

Morphological examination

The histology specimens of transplants were fixed in 10% formalin at 4°C overnight, then embedded in paraffin and cut into 5-µm sections. The sections were mounted on slides and stained with Safranin O (SO) (Pritzker et al., 2006). SO stained sections underwent morphological observation under an Olympus (1X71) microscope. For transmission electron microscopy (TEM), nude mouse were given a lethal dose of anaesthetic and perfused transcardially with 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M phosphate buffer at pH 7.3. 100 nm sections of isolated and fixed specimens were cut with a diamond knife, mounted on copper grids and visualized under TEM (Hitachi-H 7100, Tokyo, Japan).

RNA Extraction and real-time qPCR

Total RNA was extracted from the chondrocytes with the RNAeasy kit (Qiagen, Valencia, CA). The purity of total RNA was quantified by the detection of absorbance at 260/280 nm with a Du-800 UV-spectrophotometer (Beckman Coulter, Mississauga, Ontario, Canada). Total RNA (1 µg) was reverse-transcribed into cDNA, using the Advantage RT-for-PCR kit (Qiagen) according to the manufacturer's instructions. The first-strand cDNA served as templates for real-time polymerase chain reaction (RT-qPCR) using the RT-PCR detection system (DNA Engin Opticon system, Bio-Rad, CA) with specific primers (disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTs-5), aggrecan and glyceraldehyde-3-phosphate dehydrogenase [GAPDH]). These primers were designed according to how it was previously reported (Huang et al., 2004; Makihira et al., 2003) and list as follows:

ADAMTs-5: 5'-ATGACCATGAGGAGCACTACGA-3' (forward),
5'-GGAGAACATATGGTCCCAACGT-3' (reverse);
Aggrecan: 5'-ACATCCCAGAAAACTTCTTT-3' (forward),
5'- CGGCTTCGTCAGCAAAGCCA-3' (reverse);
Collagen type II: 5'- AACACTGCCAACGTCCAGAT -3' (forward),
5'- CTGCAGCACGGTATAGGTGA -3' (reverse);
GAPDH: 5'-ATCACTGCCACCCAGAAGAC-3' (forward),
5'- ATGAGGTCCACCACCCTGTT -3' (reverse).

RT-qPCR was performed under the following optimized conditions: pre-denaturation at 95°C for 5 min followed by 45 cycles of denaturation at 95°C for 20 s, annealing for 20 s and extension at 72°C for 20 s. Annealing temperature for GAPDH, ADAMTs-5 and aggrecan was 57, 60 and 62°C, respectively. All PCRs were performed in duplicates, and gene expression was normalized to that of GAPDH. PCR products were subjected to melting curve analysis, and the data were analyzed using Opticon Monitor 1.07 software.

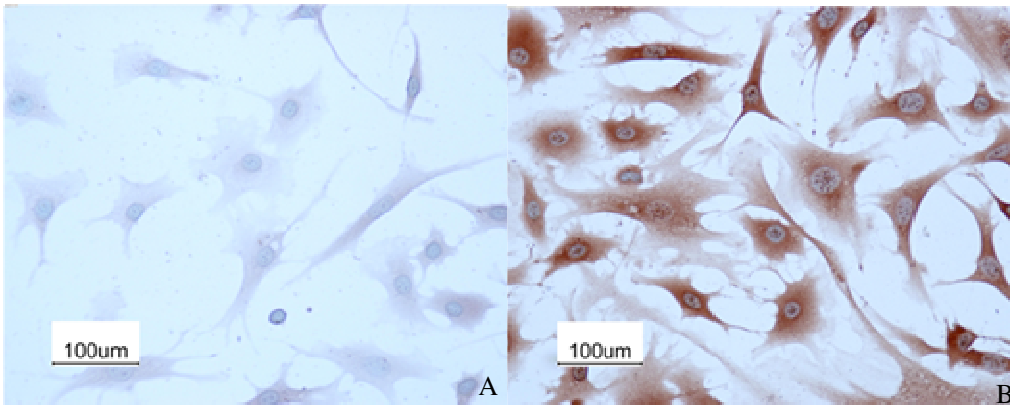

Figure 1. As compared to controls (A) omitting the primary antibody, positive staining of specific type II collagen (B) was observed for validation of chondrocytes.

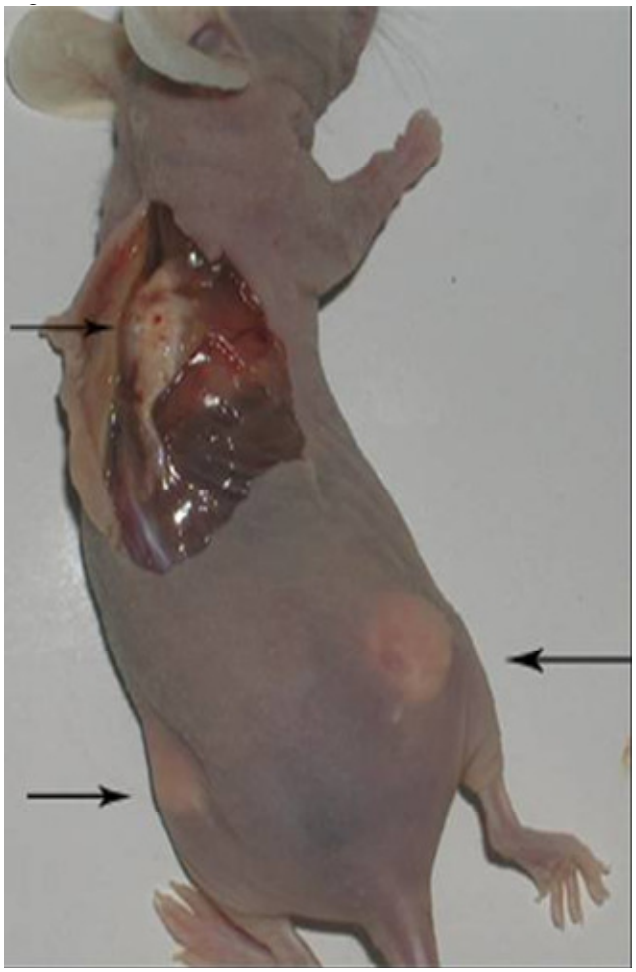

Figure 2. Cartilage-like tissues were found 6 weeks after transplantation.

Statistical analysis

Statistical analysis was performed with SPSS version 17.0 statistic software package. Repeated measures analysis of variance was used to compare the outcome scores at different time points. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Immunocytochemistry for type II collagen

Results showed chondrocytes were positive of type II collagen when compared with controls (Figure 1). This result verified that these cells were chondrocytes which could specifically secret type II collagen.

Morphological examination by SO staining and TEM

Six weeks after transplantation, cartilage-like tissues were found (Figure 2). The HBP-A was positive for SO, and the histological features of cartilage in the HBP-A group were superior to that in the alginate hydrogel alone group. Chondrocytes were evenly distributed in the ECM and round with lacunae in the scaffold. The pericellular and inter-territorial matrix region was also positive for SO, indicating the presence of proteoglycan-rich matrix (Figure 3). Cells were well-distributed within the homogenous ECM, in concert with the presence of proteoglycan-rich matrix and GAG accumulation. Both groups exhibited round chondrocyte cluster filling in several void spaces of the scaffold. In the HBP-A group, the amount of proteoglycan-rich matrix and GAG at the center was significantly higher than that in alginate alone group.

Under TEM, cells and collagens were present in the intercellular space in the superficial scaffold (Figure 4). However, the amount of collagen was higher and the arrangement of collagens was more regular in the HBP-A group than in the alginate alone group. In addition, the cells were rich in organelles. The difference between the chondrocyte-alginate construct and the HBP-A treated group was clearly visible in terms of overall cells

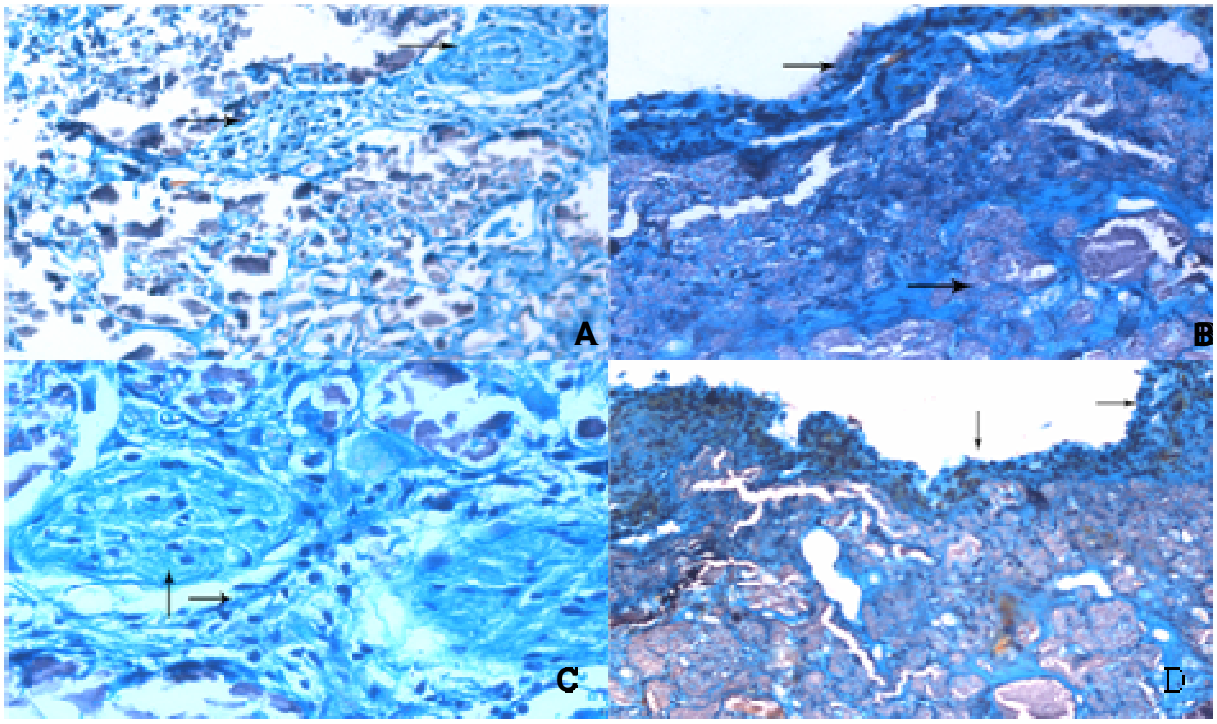

Figure 3. Chondrocytes were evenly distributed in the ECM and round. The pericellular and inter-territorial matrix region was strongly positive for SO (B.D) in HBP-A group when compared with alginate alone group (A.C), indicating the presence of proteoglycan-rich matrix

organization and collagen distribution in all specimens. In a summary, both *in vivo* and *in vitro* morphological characteristics demonstrated the presence of cartilaginous tissues, and more collagens were found in the HBP-A group than in the alginate alone group.

Effect of HBP-A on mRNA expressions of ADAMTs-5, aggrecan and type II collagen

The mRNA expressions of ADAMTs-5, aggrecan and type II collagen were not detectable in alginate only group. When chondrocytes were embedded in the alginate and HBP-A, compared with alginate only group, the expressions of type II collagenin was significantly increased and ADAMTs-5 markedly decreased ($P<0.05$) (Figure 5).

DISCUSSION

Articular cartilage has a limited capacity to self-repair after trauma. Vacanti et al. (1994) and Sakata et al. (1994) have grown tracheal epithelial cells over the engineered tissue to develop the tissue-engineered cartilage to replace small tracheal defects. Great advances have been made in recent years in the autogeneic chondrocytes, as a source for cartilage tissue engineering. This technique involves the implantation of chondrocytes in suspension or a variety of cells carrying scaffolds such as hyaluronic acid, alginate, agarose/alginate, fibrin or collagen (Tognana et al., 2007). Hydrogels are a class of polymer-based biomaterials that have been extensively used in tissue engineering as scaffolds (Sha'ban et al., 2008). Alginate hydrogel has been widely used for encapsulating chondrocytes in cartilage tissue engineering (Hwang et al., 2007), generating the cells combining with alginate hydrogel as a temporary matrix to achieve a favorable three-dimensional environment.

In our previous study, alginate was used as an injectable delivery system in the culture of chondrocytes and results showed that alginate was biocompatible with chondrocytes (Grandolfo et al., 1993). This study was to investigate the effects of glucan HBP-A on the chondrocytes seeded with alginate hydrogel. Specifically, HBP-A significantly increased the *in situ* collagen secretion of alginate hydrogel and chondrocytes system demonstrated by histological examination and PCR assay. ADAMT-5 mRNA expression in this system was also markedly decreased. Therefore, the chondrocyte biosynthetic may respond to, or may also affect the continued integrity and biomaterial property of the constructs' surrounding of host cartilage tissue in a logical reasoning.

Potential phenotypic changes are characteristics of chondrocytes. Several factors such as retinoic acid, bromodeoxyuridine and IL-1, can induce the

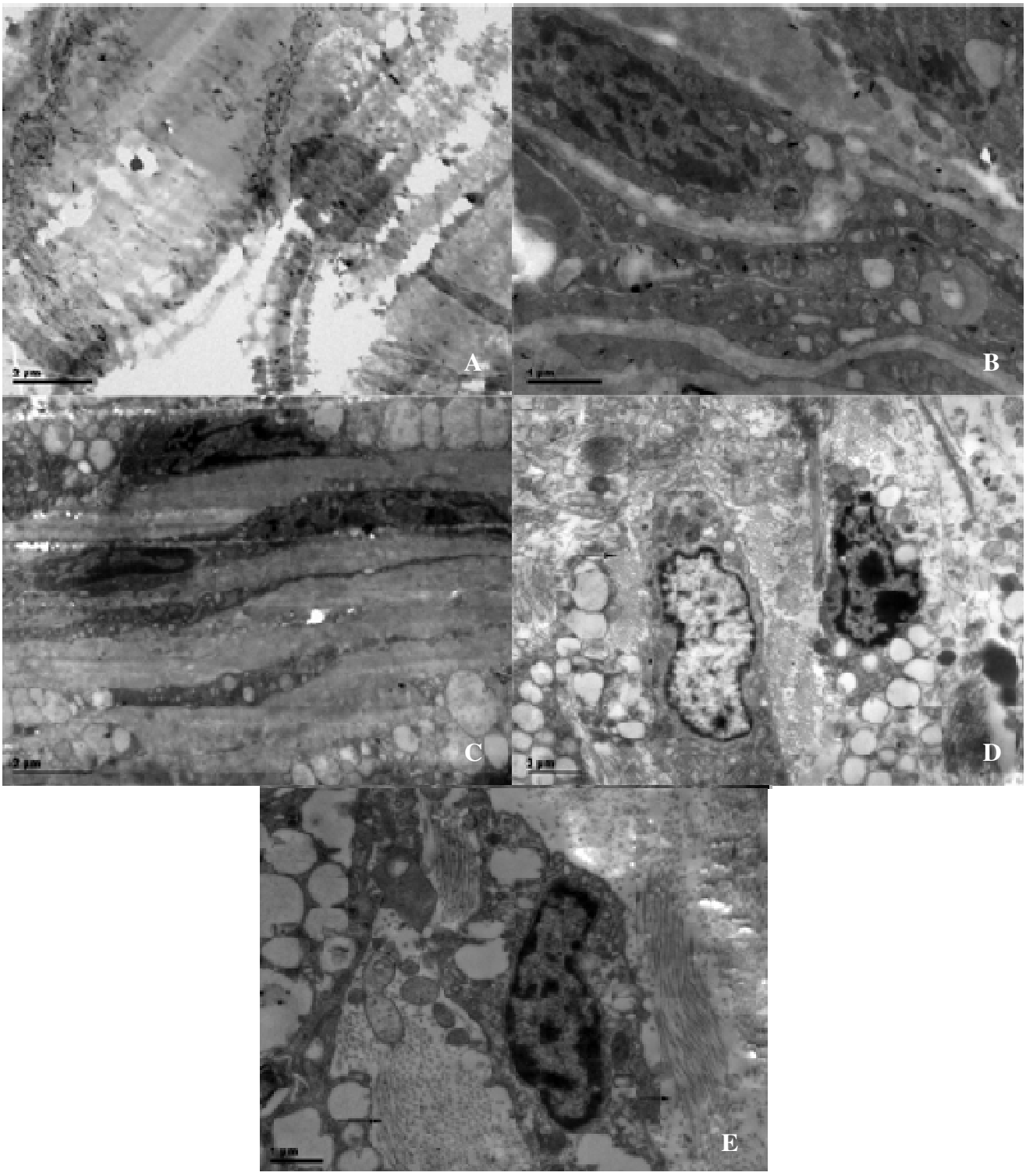

Figure 4. No chondrocyte was found in control group (alginate alone) (A). Regularly compact collagen fibers were seen in enfolding cells in alginate alone group (B,C). Chondrocytes and collagens were present in the intercellular space in HBP-A group (D,E). Moreover, the collagen amount and arrangement were more apparent than those in alginate alone group.

dedifferentiation of chondrocyte phenotype to a fibroblast-like phenotype. The chondrocytes discontinue the expression of aggrecan and type II collagen, though they are still active and express collagen types I, III, and V

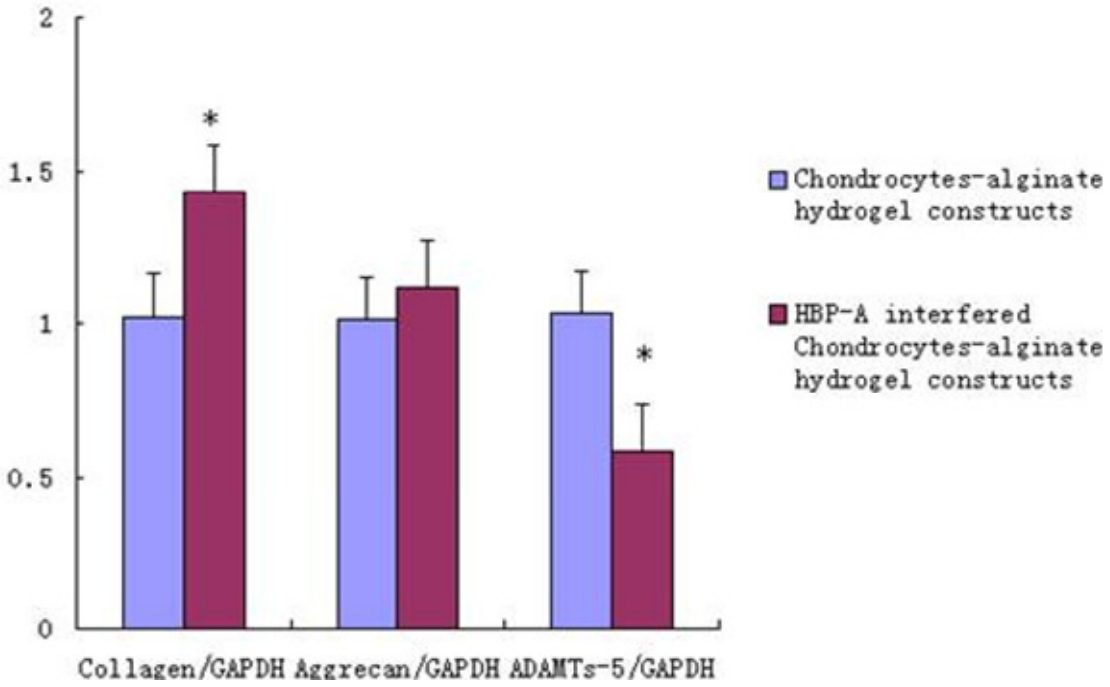

Figure 5. Six weeks after transplantation, as compared to control group, type II collagen mRNA expression was significantly increased, and ADAMTs-5 mRNA expression decreased in HBP-A group. Aggrecan mRNA expression was not different between alginate alone group and HBP-A group. Gene expression was not detectable in alginate alone group. *P<0.05 vs alginate alone group.

(Akisaka, 2003). The chondrocyte phenotypes are categorized largely by the expressions of collagen subtypes (Konttinen et al., 2005). Mature chondrocytes express typical cartilage collagen type II linking to proteins (Eyre, 2004). In this study, the primary chondrocytes were isolated from rabbit cartilage and verified by immunohistochemistry for type II collagen. In the *in vivo* experiment, collagen expression and cartilage-like tissues were confirmed by histological examination, and the mRNA expression of type II collagen was further validated by RT-PCR. This implies HBP-A may have the potential to keep phenotypic characteristics of chondrocytes in the scaffold.

In a previous study, Alcine-blue staining was used to validate the cartilage-like tissue 4 weeks after chondrocyte-alginate hydrogel injection (Xu et al., 2000). Staining of alginate hydrogel-chondrocytes with SO allows the visualization of newly synthesized matrix. In our study, histological examination of cartilaginous tissue was performed 6 weeks after implantation. Results revealed cartilage-like matrix positive for SO staining. At this time point, histological differences were significant under TEM between HBP-A group and alginate alone group. Newly formed collagen concentrated around the cells. The expression of collagen type II, cartilage-specific ECM, in the HBP-A group was noticeably superior to the control group. Clearly, this may be contributed to a more even distribution of chondrocytes in the hydrogel scaffold, which facilitates us to quantitatively detect the collagen expression *in vivo*. Another possibility resulting in increased collagen expression may be due to significantly inhibiting the catabolic enzymes, such as ADAMTs-5, since results indicated chondrocytes expressed significantly more extracellular matrix collagen and less ADAMTs-5 in the presence of HBP-A. It is currently believed that ADAMTs-4 and/or ADAMTs-5, aggrecanases from ADAMTS enzyme family, are responsible for ECM depletion. ADAMTS inhibitors currently in development are expected to show excellent specificity and the crystal structures of several ADAMTS enzymes are available to guide drug design.

In previous reports, BMPs or BMP-7 were commonly used in chondrocyte systems aiming to enhance the expressions of type II collagen and aggrecan (Nishida et al., 2004). Similar findings were also reported in the study of Fan et al. (2007), in which chondrocytes showed a higher expression of anabolic genes, collagen type II and aggrecan, after stimulation by glucosamine. In other reports, the chondrocytes exhibited a similar behaviors in the scaffolds regarding proliferation level, deposition of GAG in scaffolds and expressions of types I, II and X collagens, aggrecan, MMP-1, -13 and the integrin subunits alpha 10 and alpha 11 (Endres et al., 2007; Chajra et al., 2008). In this study, our results showed that chondrocytes were metabolically active and responded to the HBP-A treatment generating type II collagen. There was an increasing trend in aggrecan expression in *in vivo* experiment, despite it was of no statistical significance.

Several potential disease-modifying polysaccharides for OA include glucosamine, chondroitin sulfate, hyaluronan (HA), and pentosan polysulfate. Although the mechanisms of their functioning *in vivo* are unknown, results from *in vitro* and *in vivo* experiments suggest that their efficacy might be partly due to the inhibition of pro-inflammatory pathways leading to down-regulation of ADAMTs-5 (Fosang and Little, 2008). Glucosamine contributes to the chondroprotection. HA is particularly rich in the ECM of articular cartilage, in which HA exerts a number of biological effects, not only as a structural component but as an informational molecule. Moreover, HA can be synthesized in a variety of physical forms, including hydrogels, sponges, fibers and fabrics, whichallow the development of a variety of hyaluronan-based scaffolds. HA was also found to have the capacity to penetrate and disengage cell-matrix interactions of chon- drocytes within embryonic chick tibial cartilage (Knudson et al., 2000). Similar to glucosamine and HA, alginate as well as HBP-A are a kind of polysaccharides contributing to the composition of ECM. Differently, HBP-A is a kind of glucan and has been extracted by our group. Whether alginate exerts synergistic effect with HBP-A is unclear. Given the reason that polysaccharides have various forms, the mechanisms of different forms functioning may differ.

In summary, HBP-A could inhibit the expression of ADAMT-5, in the chondrocytes of the constructs. The chondrocyte were verified by histological examination and molecular study and results showed collagen synthesis was more active in the presence of HBP-A. Therefore, the HBP-A in chondrocyte can be used as a target to inhibit related catabolic enzymes and promote collagen expression. Our results provide a possible approach to develop a tissue-engineered or chondroprotective agent. Further studies are needed to improve the biological or biomechanical properties of the HBP-A modified implants, which may be more suitable for surgical transplantation.

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REFERENCES

Akisaka T (2003). Cytoskeleton of cultured osteoclasts on bone slices and glass. Clin. Calcium., 13:449-454

Chajra H, Rousseau CF, Cortial D, Ronzière MC, Herbage D, Mallein-Gerin F, Freyria AM (2008). Collagen-based biomaterials and cartilage engineering. Application to osteochondral defects. Biomed. Mater. Eng.,18:S33-45

Chubinskaya S, Merrihew C, Cs-Szabo G, Mollenhauer J, McCartney J, Rueger DC, Kuettner KE (2000). Human articular chondrocytes express osteogenic protein-1. J. Histochem. Cytochem., 48:239-250

Endres M, Neumann K, Schröder SE, Vetterlein S, Morawietz L, Ringe J, Sittinger M, Kaps C (2007). Human polymer-based cartilage grafts for the regeneration of articular cartilage defects. Tissue. Cell., 39: 293-301

Eyre DR (2004). Collagens and cartilage matrix homeostasis. Clin. Orthop. Relat. Res., 427(Suppl): S118-122

Fan Z, Söder S, Oehler S, Fundel K, Aigner T (2007). Activation of interleukin-1 signaling cascades in normal and osteoarthritic articular cartilage. Am J Pathol., 171: 938-946

Fosang AJ, Little CB (2008). Drug Insight: aggrecanases as therapeutic targets for osteoarthritis. Nat. Clin. Pract. Rheumato., 14: 420-427

Feng W, Shi YY, Shen PZ (2004). The study of Chinese medicine on the proliferation of chondrocytes in vitro by MTT method. Acta. Universitatis. Traditionis. Medicalis. Sinensis. Pharmacologiaeque. Shanghai., 4: 43-45

Grandolfo M, D'Andrea P, Paoletti S, Martina M, Silvestrini G, Bonucci E, Vittur F (1993). Culture and differentiation of chondrocytes entrapped in alginate gels. Calcif. Tissue. Int., 52: 42-48.

Huang CY, Hagar KL, Frost LE, Sun Y, Cheung HS (2004). Effects of cyclic compressive loading on chondrogenesis of rabbit bone-marrow derived mesenchymal stem cells. Stem. Cells., 22: 313-323

Hui W, Cawston T, Rowan AD (2003). Transforming growth factor beta 1 and insulin-like growth factor 1 block collagen degradation induced by oncostatin M in combination with tumor necrosis factor α from bovine cartilage. Ann. Rheum. Dis., 62: 172-174

Hunziker EB (2002). Articular cartilage repair: basic science and clinical progress. A review of the current status and prospects. Osteoarthritis. Cartilage., 10: 432-463

Hwang NS, Varghese S, Elisseeff J (2007). Cartilage tissue engineering: Directed differentiation of embryonic stem cells in three-dimensional hydrogel culture. Methods. Mol. Biol., 407:351-373

Knudson W, Casey B, Nishida Y, Eger W, Kuettner KE, Knudson CB (2000). Hyaluronan oligosaccharides perturb cartilage matrix homeostasis and induce chondrocytic chondrolysis. Arthritis. Rheum., 43: 1165-1174

Konttinen YT, Ma J, Ruuttila P (2005). Chondrocyte-mediated collagenolysis correlates with cartilage destruction grades in osteoarthritis. Clin. Exp. Rheumatol., 23: 19-26

Makihira S, Yan W, Murakami H, Furukawa M, Kawai T, Nikawa H, Yoshida E, Hamada T, Okada Y, Kato Y (2003). Thyroid hormone enhances aggrecanase-2/ADAM-TS5 expression and proteoglycan degradation in growth plate cartilage. Endocrinol., 144: 2480-2488

Monfort J, Pelletier JP, Garcia-Giralt N, Martel-Pelletier J (2008). Biochemical basis of the effect of chondroitin sulphate on osteoarthritis articular tissues. Ann. Rheum. Dis., 67: 735-740

Nishida Y, Knudson CB, Knudson W (2004). Osteogenic Protein-1 inhibits matrix depletion in a hyaluronan hexasaccharide-induced model of osteoarthritis. Osteoarthritis. Cartilage., 12: 374-382

Pritzker KP, Gay S, Jimenez SA, Ostergaard K, Pelletier JP, Revell PA, Salter D, van den Berg WB (2006). Osteoarthritis cartilage histo-pathology: grading and staging. Osteoarthritis. Cartilage., 14: 13-29

Sakata J, Vacanti CA, Schloo B, Healy GB, Langer R, Vacanti JP (1994). Tracheal Composites tissue engineered from chondrocytes, tracheal epithelial cells, and synthetic degradable scaffoding. Transplant. Proc., 26: 3309-3310

Schneiderman R, Rosenberg N, Hiss J, Lee P, Liu F, Hintz RL, Maroudas A (1995). Concentration and size distribution of insulin-like growth factor-1 in human normal and osteoarthritic synovial fluid and cartilage. Arch. Biochem. Biophys., 324: 173-188

Sha'ban M, Kim SH, Idrus RB, Khang G (2008). Fibrin and poly(lactic-co-glycolic acid) hybrid scaffold promotes early chondrogenesis of articular chondrocytes: an in vitro study. J. Orthop. Surg., 25: 3-17

Tognana E, Borrione A, De Luca C, Pavesio A (2007). Hyalograft C: hyaluronan-based scaffolds in tissue-engineered cartilage. Cells. Tissues. Organs., 186: 97-103

Vacanti CA, Paige KT, Kim WS, Sakata J, Upton J, Vacanti JP (1994). Experimental tracheal replacement using tissue-engineered cartilage. J. Pediatr. Surg., 29: 201-205

Wu K, Wen SF, Huang L (2004). In vitro effect of a Traditional Chinese Medicine formula-BYYNG on breast cancer cell line-4T1 for prevention of bone metastasis. Hong. Kong. J. Orthop., 8(S):77

Xu H, Hu YY, Liu SB, Lao (2000). Experimental study of *in vitro* culture of chondrocytes combined with injectable calcium alginate gel for neo-cartilage tissue engineering. Chin. J. Orthoap., 20(9): 533-535.