Conditioned Medium from Chondrocyte/Scaffold Constructs Induced Chondrogenic Differentiation of Bone Marrow Stromal Cells

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ABSTRACT

For the application of bone marrow stromal cells (BMSCs) in cartilage tissue engineering, it is imperative to develop efficient strategies for their chondrogenic differentiation. In this study, the conditioned media derived from chondrocyte/scaffold constructs were used to direct chondrogenic differentiation of BMSCs. The porcine articular chondrocytes were seeded on the PGA/PLA scaffolds to form chondrocyte/scaffold constructs and were cultured to form engineered cartilage *in vitro*. The culture media were collected as conditioned media and used for chondrogenic induction of BMSC pellets (experimental group, Exp.). The chondrocyte pellets and BMSC pellets were cultured routinely as positive control (PC) and negative control (NC), respectively. After 4 weeks, the wet weight and GAG content in Exp. group and PC group were significantly higher than that in NC group. Histological and immunohistochemical analysis showed that cartilaginous tissue was formed with typical cartilage lacuna structure and positive staining of collagen Type II (Col II) in the peripheral area of the BMSC pellets in Exp. group. Gene expression of *Sox9*, *Col II*, and *COMP* in Exp. group and PC group were significantly higher than that in NC group. The growth factors in the conditioned media derived from human costal chondrocytes-scaffold constructs were tested by protein microassay. The conditioned media contained low levels of TGF-β1,2,3, IGF-1 and high levels of IGF-2, FGF-4, and IGFBP4,6, and so forth. The soluble factors derived from the engineered cartilage can induce chondrogenic differentiation of BMSCs independently. Many cytokines may function in chondrogenesis in a coordinated way. Anat Rec, 295:1109–1116, 2012.

Key words: bone marrow stromal cells; conditioned medium; chondrogenesis; chondrocyte/scaffold construct; protein microarray; soluble factors

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In vivo niche plays an important role in directing the committed differentiation of stem cells (Liu et al., 2009). In articular osteochondral defects, noninduced BMSCs could form satisfactory cartilage and subchondral bone tissue (Zhou et al., 2010). However, in subcutaneous environments, BMSCs only developed into vascularized fibrous tissue (Zhou et al., 2005). These results indicated that the niche of cartilage played an important role in determining chondrogenesis of BMSCs.

Chondrocyte is the major cell type in cartilage and is responsible for development and maintenance of the articular niche (Lin et al., 2006; Liu et al., 2010). Studies by Yang et al. (2009) revealed that cotransplantation of BMSCs, chondrocytes, and chondrogenic factors in a hydrogel could enhance the chondrogenesis of BMSCs in subcutaneous environments. Another study showed that micromass coculture of human articular chondrocytes and human bone marrow mesenchymal stem cells with or without growth factors could form stable neocartilage tissue *in vitro* (Giovannini et al, 2010). Moreover, Liu et al. (2010) found that chondrocytes alone could create a chondrogenic niche in nonchondrogenic sites to direct chondrogenesis of stem cells. In these coculture models, chondrocytes recapitulated the articular chondrogenic niche and promoted chondrogenesis of BMSCs, and paracrine soluble factors secreted by chondrocytes might be a major mechanism to induce chondrogenesis of BMSCs (Yang et al., 2009; Liu et al., 2010). To investigate whether the soluble factors could induce chondrogenesis of BMSCs independently, we used the conditioned medium derived from chondrocyte/scaffold constructs to induce chondrogenic differentiation of BMSC pellets. Furthermore, the growth factors in the conditioned media were also characterized by protein microarray.

MATERIALS AND METHODS

Animals

Eight-week-old pigs were obtained from the Institute of Animal Science at the Chinese Agricultural University. The pigs were kept under conventional conditions with free access to water and a regular supply of food. All the animals were operated in accordance with the guidelines for animal experiments approved by Peking Union Medical College and Plastic Surgery Hospital.

Isolation, Culture of BMSCs, and Chondrocytes

The pigs were sedated and anesthetized with xylazine (2 mg kg^{-1}) and ketamine (20 mg kg^{-1}) injected intramuscularly. Bone marrow samples were obtained by iliac crest aspiration according to previously established methods (Zhou et al., 2006). Briefly, the bone marrow aspirate (10 mL) was combined with 40 mL of Dulbecco's Modified Eagle Medium (DMEM; Gibco, USA) and centrifuged at 1,500 rpm for 5 min. The supernatant was removed and the cells were washed repetitively for two times and resuspended in DMEM supplemented with 10% (v/v) fetal bovine serum (FBS; Gibco, USA), 100 U mL^{-1} penicillin and 0.1 mg mL^{-1} streptomycin (Gibco, USA). Primary cells were seeded on a culture dish at a density of 5×10^5 nucleated cells cm^{-2} and cultured at 37°C with 5% CO_2. After 4 days, nonadherent cells were removed by washing with phosphate buffer saline (PBS; Gibco, USA) and remaining adherent cells were further cultured with medium changed every 3 days. The adherent cells were cultured until cell clones reached over 80% confluence and then were digested with 0.25% (w/v) trypsin plus 0.02% (w/v) EDTA and subcultured at a density of 1×10^4 cells cm^{-2} in a new plate. Then, BMSCs were expanded to Passage 3 for testing.

Cartilage tissue was harvested in sterile conditions from the articular cartilage of the pigs that were euthanized with an overdose of Pentobarbital (100 mg kg^{-1} IV). According to previously described method (Liu et al., 2007), the articular cartilage was cut into $2 \times 2 \times 1$ mm^3 slices and washed twice with PBS. After being digested with 0.3% (w/v) collagenase II (Sigma, USA) at 37°C for 2 h, the cartilage slices were further digested with 0.1% (w/v) collagenase II for 12–16 h. Then, chondrocytes were harvested, counted, and seeded onto culture dishes at a cell density of 2.5×10^4 cells cm^{-2} for culture and subculture in regular media. The chondrocytes were expanded to Passage 1 for further experiments.

Preparation of Chondrocyte/Scaffold Constructs and Collection of Conditioned Medium

Polyglycolic acid (PGA) scaffold was prepared as previously described (Liu et al., 2007). Briefly, 25 mg of PGA (321 kD) unwoven fibers (Albany International Research, Albany, USA) were compressed into a cylinder shape with 12-mm diameter and 2-mm thickness (Fig. 1A). To solidify the scaffold shape, 300 μL of 1% PLA (80 kD, Sigma, USA) diluted in dichloromethane was used to coat the PGA fibers. The scaffolds were sterilized by soaking in 75% alcohol and washed three times with PBS followed by two washes with DMEM.

Chondrocytes (Passage 1) were seeded into the PLA/PGA scaffolds (1.0×10^7 cells in 0.3 mL $scaffold^{-1}$) to form chondrocyte/scaffold constructs and cultured in DMEM with 10% FBS, 100 U mL^{-1} penicillin, and 0.1 mg mL^{-1} streptomycin. Chondrocyte/scaffold constructs were cultured in a 5% CO_2 incubator (Thermo, USA) at 37°C with culture medium changed every 3 days.

After the chondrocytes/scaffold constructs were cultured for 1 week, half of the culture media of chondrocyte–scaffold constructs began to be changed every day and 3 mL of the culture medium was collected every time, centrifuged at 600*g* for 8 min at 20°C. The supernatant was filtered using a 0.22 mM filter and was used as conditioned medium for induction of BMSC pellets (experiment group, as showed below).

Pellets Culture and Grouping Design

Pellet culture system was performed using method previously outlined by Johnstone et al. (1998). Aliquots of 5×10^5 cells were centrifuged at 1500 rpm for 5 min in 15 mL polypropylene conical tubes. Pelleted cells were incubated at 37°C under 5% CO_2 with loosened caps to permit gas exchange. After 24 h of incubation, the sedimented cells formed a spherical aggregate at the bottom of each tube. BMSC pellets cultured in conditioned medium (mentioned above) were used as experiment group (Exp., $n = 6$). First passage chondrocytes were pelleted and cultured in routine media as

positive control group (PC, n = 6). BMSC pellets cultured in routine culture media were used as negative control group (NC, n = 6). Culture media were changed every day and pellets in the three groups were harvested after 4 weeks.

Wet weight and glycosaminoglycan (GAG) content of the pellets. After 4 weeks of culture, the wet weight of the specimens was tested and the glycosaminoglycan (GAG) content was assayed by Alcian Blue colorimetric analysis as previously described (Zhou et al., 2006).

Histology and Immunohistochemistry Analysis of the Pellets

The chondrocyte/scaffold constructs and the pellets in all groups were fixed, embedded in paraffin, and cut into 5-μm sections. The sections were stained with hematoxylin and eosin (HE) and Safranin O to evaluate histological structure and deposition of the cartilage specific matrix. Expression of Type II collagen was detected by a mouse anti-collagen-II monoclonal antibody (IgG, Santa Cruz, CA), and a horseradish peroxidase (HRP)-conjugated anti-mouse antibody (DAKO, Carpinteria, CA) followed by color development with diaminobenzidine tetrahydrochloride (DAB).

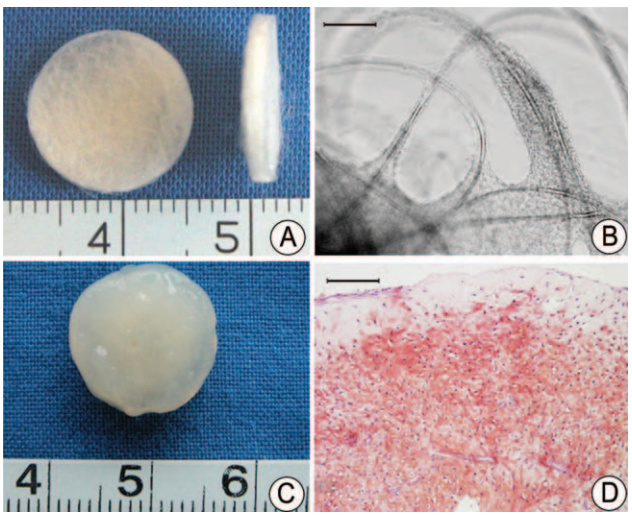


Fig. 1. PGA/PLA scaffolds and chondrocyte/scaffold constructs. (**A**) Gross view of PGA/PLA scaffold; (**B**) Microscope examination of chondrocyte/scaffold construct cultured for 1 week; (**C**) Gross view of the chondrocyte/scaffold construct cultured for 5 weeks; (**D**) Safranin O staining of the chondrocyte/scaffold construct cultured for 5 weeks. Scale bar = 100 μm.

Quantitative Reverse-Transcription Polymerase Chain Reaction

Expression of cartilage-specific markers, such as collagen Type II (*ColII*), collagen Type II (*ColII*), cartilage oligomeric matrix protein (COMP), and SRY-box containing gene 9 (*ox9*), were analyzed by quantitative real-time PCR. The primer sequences for these porcine genes used in the study are shown in Table 1. Primer sequences were designed using Gene Runner software. The specificity of sequences was verified using the basic local alignment search tool (BLAST) of the National Center for Biotechnology Information (NCBI) online database.

A LightCycler® 480 PCR machine was used for PCR cycling. The PCR mixture contained 100 ng cDNA, 400 nM PCR primer, and Power SYBR Green PCR Master Mix (CWBIO, CHN). The PCR reaction profile consisted of predenaturation for 2 min at 94°C, and amplification for 45 cycles at 94°C for 15 sec and 60°C for 30 sec. The series of cycles were followed by a melt curve analysis to ensure reaction specificity. The expression level of each gene was normalized to GAPDH. Subsequently, the expression level of the control group was set as "one" and expressions of target genes in experimental groups relative to that of the control group were calculated using the $2^{-(\Delta\Delta Ct)}$ method.

Enzyme-Linked Immunosorbent Assay (ELISA) and Human Growth Factor Antibody Array

Human chondrocytes were isolated from the spare human costal cartilage of two patients with microtia undergoing ear reconstruction. Patients were 7 and 9 years old, respectively and written informed consent was obtained from all participants. Isolation, culture of chondrocytes, and preparation of chondrocytes–scaffold constructs were the same as methods mentioned above. chondrocytes–scaffold constructs were cultured in serum-containing DMEM for 1 week. Then, culture medium were changed by serum-free medium (low-glucose DMEM with 1% ITS, 10^{-7} M Dexamethasone, 50 μg mL^{-1} Vitamin C) for another 3 days. At the end of the third day, the conditioned serum-free media were analyzed with RayBio® human Growth Factor antibody array I(Ray Biotech, Norcross, GA). According to the

TABLE 1. Primer sequences of quantitative reverse transcription-PCR

Gene	Accession no.	Primer 5′–3′	PCR product size
Col II	XM_001925959.4	*F 5-TCCTGGTGAAGATGGTCGC-3* *R 5-AGCACCTGTCTCGCCATCT-3*	181 bp
COMP	XM_003123527.1	*F 5-GCCTGTGACGACGATGATG-3* *R 5-TTGTCTACCACCTTGTCCG-3*	149 bp
ColII	XM_003121273.2	*F 5-TACAGTGGTTTCCTTACAGC-3* *R 5-TTTGCTTTCACACAGAGGAG-3*	112 bp
Sox9	NM_213843.1	*F 5-CTACACAGACCACCAGAAC-3* *R 5-GTCTTTCTTCGGTGGGTTAC-3*	271 bp
GAPDH	NM_001206359.1	*F 5- CTGCCCCTTCTGCTGATGC -3* *R 5-TCCACGATGCCGAAGTTGTC -3*	151 bp

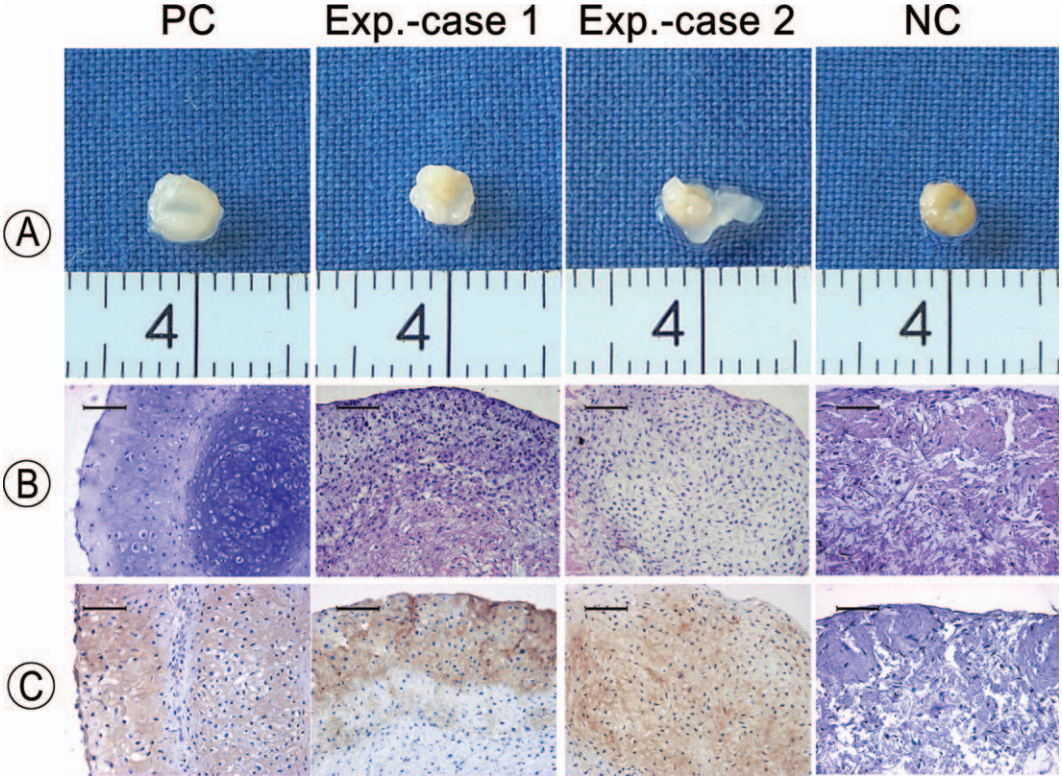


Fig. 2. (**A**) Gross view of pellets in PC group (chondrocyte pellet), Exp. group (BMSC pellet induced by conditioned media) and NC group (BMSC pellet without induction); (**B**) HE staining of the pellets in all groups. Histology showed formation of lacuna structure in PC group and in peripheral area of Exp. group. No cartilage-like tissue was formed in NC group; (**C**) Col II immunohistochemical evaluation of the pellets in all groups. Expression of Col II was shown in PC group and in peripheral area of Exp. group. No Col II was expressed in NC group. Scale bar = 100 μm.

manufacturer's protocol, the membranes were incubated in blocking buffer for 30 min, followed by overnight incubation at 4°C with conditioned media. Membranes were washed five times with wash buffer and incubated for 2 h with biotin-conjugated antibodies to each membrane. Then, membranes were washed five times with wash buffer and incubated for 2 h with horseradish peroxidase-conjugated streptavidin to each membrane. After the washing process, the human growth factors were detected by enhanced chemiluminescence reagents.

Transforming growth factor beta1(TGF-β1) and insulin-like growth factor 1 (IGF-1) in the conditional media were quantitatively detected by ELISA using human TGF-β1 immunoassay (DB100B, Quantikine, R&D) and human IGF-1 immunoassay (DG100, Quantikine, R&D). Insulin-like growth factor binding protein 4 (IGFBP-4) and insulin-like growth factor binding protein 6 (IGFBP-6) were quantitatively detected by ELISA using RayBio® Human IGFBP-4 ELISA Kit and RayBio® Human IGFBP-6 ELISA Kit.

Statistical Analysis

Data was presented as mean ± standard error of the mean. The difference of the data between groups was evaluated by one-way analysis of variance (ANOVA). Analysis was performed with SPSS 11.0 software and a P value <0.05 was considered statistically significant.

RESULTS

Evaluation of the Chondrocytes and Chondrocyte/Scaffold Constructs

Chondrocytes were round- or polygon shaped in monolayer culture and attached well to the PGA/PLA scaffolds with abundant extracellular matrix at 1 week (Fig. 1B), indicating good biocompatibility between the cells and the scaffolds. After 5 weeks, the chondrocyte/scaffold constructs formed homogeneous cartilage-like tissue (Fig. 1C) with typical lacuna structure and cartilage-specific matrix deposition as shown by Safranin O staining (Fig. 1D).

Gross View and Histological Evaluation of the Pellets

After culturing for 4 weeks, the chondrocyte pellets developed into white, disc-like, smooth, and elastic cartilage-like tissue (Fig. 2A, PC). Comparing with the chondrocyte pellets, the BMSC pellets cultured in the conditioned media were relatively irregular in shape, smaller in size, and formed cartilage-like tissue only in the peripheral area (Fig. 2A, Exp. Case 1 and 2). While, the BMSC pellets cultured in routine culture medium shrunk gradually and became yellowish and inelastic tissue (Fig. 2A, NC).

Chondrocyte pellets (PC) formed homogenous cartilage-like tissue with typical lacuna structure and strong expression of ColII(Fig. 2B,C, PC). While, the BMSC pellets induced by conditioned medium (Exp.) formed heterogeneous tissue with lacuna-like structure and positive staining of ColII in the peripheral area, but not in the central area (Fig. 2B,C, Exp. Case 1 and 2). For the BMSC pellets cultured in routine medium (NC), only fibrous tissue were observed and no expression of collagen II as well, indicating that there was no spontaneous chondrogenesis occurring without chondrogenic induction (Fig. 2B,C, NC).

Wet Weight and GAG Content of the Pellets

Quantitative analysis showed that wet weight in PC group and Exp. group was higher than that in NC group ($P < 0.01$). GAG content in Exp. group reached 73% of that in PC group and was significantly higher than that in NC group ($P < 0.01$) (Fig. 3), indicating that the conditioned media from the chondrocyte/scaffold constructs induced GAG deposition in the BMSC pellets.

Quantitative gene expression of BMSCs pellets cultured in the conditioned medium. The mRNA expression levels of *Col II* and *Sox9*, which are cartilage-specific genes, in PC group and Exp. group were significantly higher than that of NC group ($P < 0.01$, Fig. 4). The expression of *Col II* in Exp. group reached 62% of that in PC group and the expression of *Sox9* in Exp. group reached 95% of that in PC group. The expression of *COMP* and *Col* IX in Exp. group was not as high as *Col II* and *Sox9*. Although there was significant difference between Exp. group and NC group in *COMP*, there was no significant difference in *ColIX*, which was related with the mechanical properties of engineered cartilage *in vitro* (Yan, et al., 2009).

Results of Human Growth Factor Antibody Array and Concentration of TGFβ1, IGF-1, and IGFBP-4,6 in the Conditioned Medium

Costal chondrocytes were expanded to Passage 1 and were seeded into the PLA/PGA scaffolds (12 mm in diameter and 2 mm in thickness) to form chondrocytes–scaffold constructs. The chondrocytes adhered to the PGA/PLA scaffolds and abundant extracellular matrix was observed under an inverted phase contrast microscope at Week 1 (Data not shown). Forty one human growth factors were tested, and we found that chondrocyte/scaffold constructs produced high levels of IGFBP-4,6, IGF-2, FGF-4, and FGF-basic, and so forth, while TGFβ1,2,3 and IGF-1 were expressed in a low level (Fig. 5).

ELISA results showed that concentrations of TGFβ1 and IGF-1 were 1,000 ± 141 pg mL^{-1} and 190 ± 17 pg mL^{-1}, respectively in the conditioned medium. Concentration of IGFBP-4 and IGFBP-6 were 10,506 ± 101 pg mL^{-1} and 5,508 ± 94 pg mL^{-1}, respectively. These results were consistent with that of human growth factor antibody array.

DISCUSSION

In the study, we used the conditioned medium derived from chondrocytes–scaffold constructs to induce chondrogenic differentiation of BMSCs pellets and provided direct evidence that chondrocytes-derived soluble factors can induce chondrogenesis of BMSCs independently. What is more, we tested some important growth factors in the conditioned medium and proposed some possible candidates responsible for chondrogenesis by means of ELISA and protein microarray.

During chondrogenic induction of BMSCs pellets, three-dimensional culture, while not monolayer culture of chondrocytes, is very important for chondrogenic induction of BMSCs. Our preliminary experiment had failed to induce chondrogenic differentiation of BMSCs using conditioned medium from monolayer-cultured chondrocytes (data not shown). Chondrocytes in monolayer culture were inclined to be dedifferentiated, expressing Type I collagen and seldom producing cartilage-specific matrix (Chen et al., 2003). Whereas, chondrocytes in three-dimensional culture were associated with differentiated phenotype, expressing Type II collagen and producing cartilage-specific matrix, and the mature process of the chondrocyte/scaffold constructs *in vitro* was similar to the process of cartilage development *in vivo* (Ichiro et al., 2002). The cytokines and growth factors secreted from the chondrocyte/scaffoled constructs might be similar to those needed in chondrogenesis. In the study, PGA/PLA material was used as the 3D scaffold for the chondrocytes. PGA-based scaffolds and PLA-based scaffolds and their patterned scaffolds were the most widely used biomaterial in cartilage tissue engineering. A large number of studies have proven that PGA or PLA-based scaffolds can successfully improve the formation of engineered cartilage (Cao et al., 1997; Carver et al., 1999; Satdanha et al., 2000). Comparing with natural biopolymeric scaffolds, PGA/PLA scaffolds have been shown to promote proteoglycan synthesis at higher rates than hyaluronan- and collagen-based natural biopolymeric scaffolds (Grande et al., 1997).Cartilage engineered with the PGA/PLA scaffold has cartilage structure and specific matrices similar to the native tissue, which ensures the secretion of effective cytokines and growth factors (Moran et al., 2003; Cui et al., 2009; Yan et al., 2009). What is more, PGA/PLA scaffolds present advantages of good flexibility and processability into different size and shapes, and the physical–chemical properties of such polymers can be easily modified, and the mechanical behavior and degradation can be suitably modulated by varying the chemical composition of the macromolecule (Cheung et al., 2007).

In our previous study, we had examined the concentration of the main factors such as TGF-β, IGF, and BMP in the culture medium of chondrocytes. The concentrations of these factors showed a time-dependent increase within 4 weeks of *in vitro* culture (Liu et al., 2010). So, in the present study, the conditioned medium was collected for 4 weeks after chondrocytes were seeded on the biodegradable scaffold. We changed half of the medium every day, and used the supernatant as conditioned medium for induction of BMSCs without delay. This process not only maintained the stability of the soluble factors, but also gave a sufficient nutritional supply to the BMSCs, which ensured the inductive effects of the soluble factors derived from chondrocytes.

As the unique cell type in cartilage tissue, chondrocytes play an important role in articular

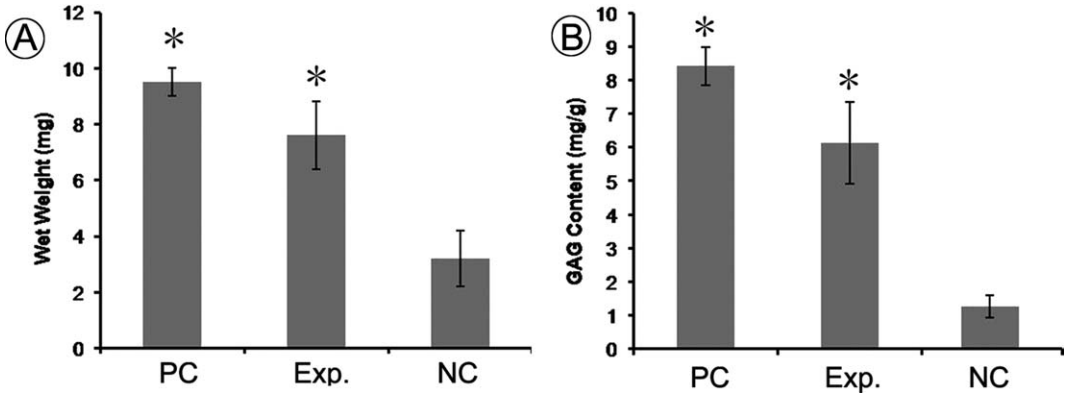


Fig. 3. (**A**) Wet weight of the pellets in the three groups. Wet weight in PC group and Exp group was significantly higher than that in NC group; (**B**) GAG content of the pellets in the three groups. GAG content in PC group and Exp group was significantly higher than that in NC group (*, comparing with NC group, $P < 0.01$, n = 4).

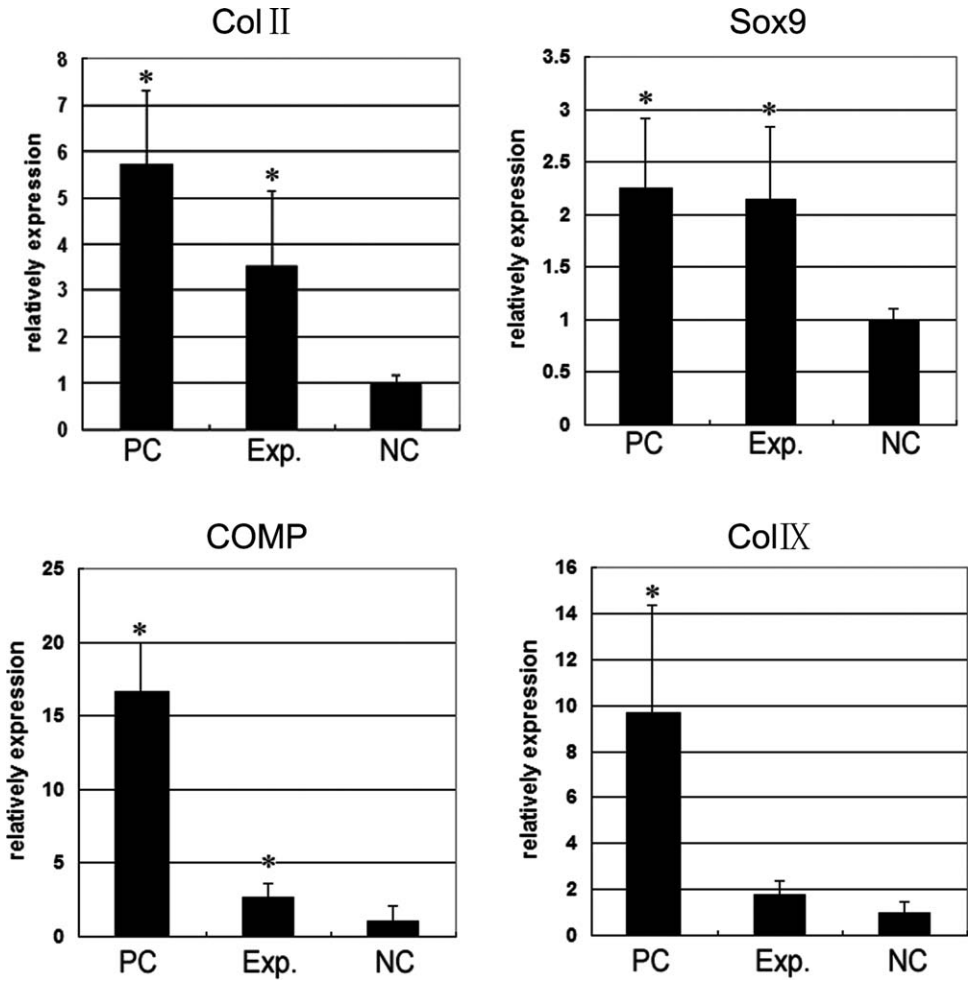
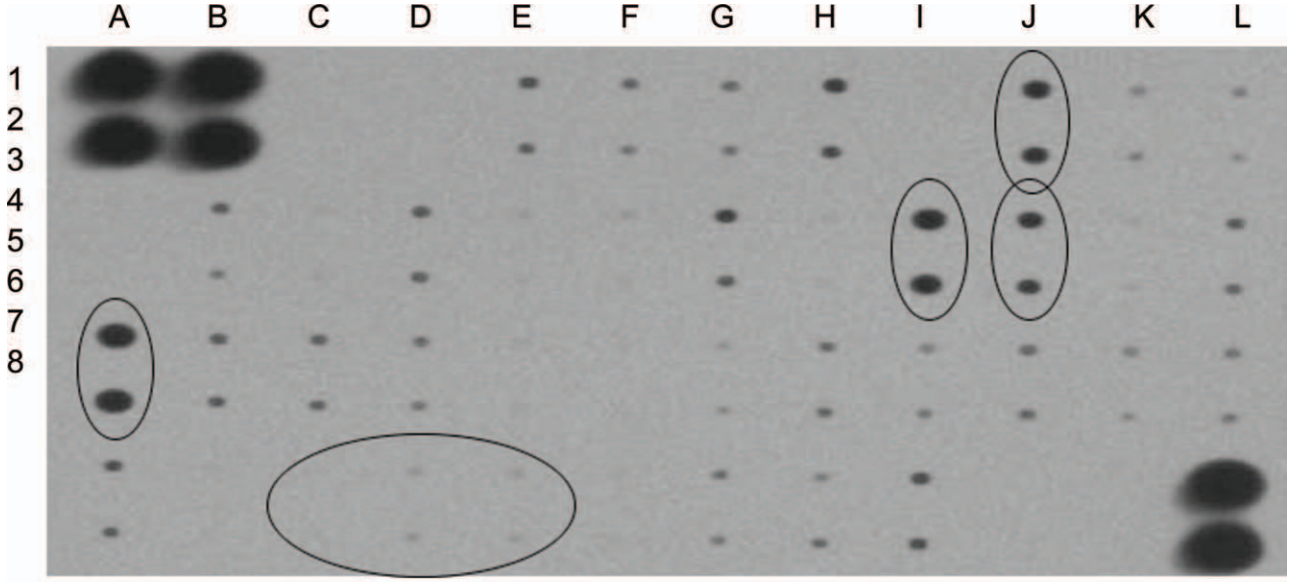


Fig. 4. Quantitative gene expression of *ColII*, *Sox9*, *COMP* and *ColIX* in PC group, Exp. Group, and NC group. (*, comparing with NC group, $P < 0.05$).

microenvironment (Muir et al., 1995). Chondrocytes can secrete a variety of growth factors including TGFβ1,3, IGF-1, fibroblast growth factors (FGFs), Bone morphogenetic proteins (BMPs), and parathyroid hormone-related protein (PTHrP), (Fischer et al., 2010), and so forth, all of which have modulatory effects on proliferation and differentiation of chondrocytes via autocrine or paracrine ways (Goldring et al., 2006). Supplementation of TGF-β



	A	B	C	D	E	F	G	H	I	J	K	L
1	POS	POS	NEG	NEG	AR	FGF basic	beta NGF	EGF	EGF R	FGF-4	FGF-6	FGF-7
2	POS	POS	NEG	NEG	AR	FGF basic	beta NGF	EGF	EGF R	FGF-4	FGF-6	FGF-7
3	GCSF	GDNF	GM-CSF	HB-EGF	HGF	IGFBP-1	IGFBP-2	IGFBP-3	IGFBP-4	IGFBP-6	IGF-I	IGF-I SR
4	GCSF	GDNF	GM-CSF	HB-EGF	HGF	IGFBP-1	IGFBP-2	IGFBP-3	IGFBP-4	IGFBP-6	IGF-I	IGF-I SR
5	IGF-II	M-CSF	M-CSF R	NT-3	NT-4	PDGF R alpha	PDGF R beta	PDGF-AA	PDGF-AB	PDGF-BB	PlGF	SCF
6	IGF-II	M-CSF	M-CSF R	NT-3	NT-4	PDGF R alpha	PDGF R beta	PDGF-AA	PDGF-AB	PDGF-BB	PlGF	SCF
7	SCF R	TGF-alpha	TGF-beta 1	TGF-beta 2	TGF-beta 3	VEGF	VEGF R2	VEGF R3	VEGF-D	BLANK	BLANK	POS
8	SCF R	TGF-alpha	TGF-beta 1	TGF-beta 2	TGF-beta 3	VEGF	VEGF R2	VEGF R3	VEGF-D	BLANK	BLANK	POS

Fig. 5. The conditioned medium was collected and analyzed by human growth factor antibody array. IGF-2, IGFBP-4,6, and FGF-4 were produced at a high level, while TGFβ1,2,3 and IGF-1 were hardly detected.

and/or IGF-1 in the culture system *in vitro* can provide an extracellular signal for directional differentiation of BMSCs to cartilage (Tuli et al., 2002; Zhou et al., 2004). In these culture systems, the concentrations of TGF-β and IGF-1 were ordinarily 10,000 and 50,000 pg mL^{-1}, respectively. However, in the present study, TGF-β1,2,3 and IGF-1 were found to be in a low level in the conditioned media, while IGF-2, FGF-4, and IGFBP-4,6 were found to be in a high level by analysis of ELISA and protein microarray. Therefore in the physiological condition, the chondrocytes secrete many cytokines and growth factors and were supposed to promote chondrogenesis in a coordinated way (Liu et al., 2010). IGF-2 level was about five times those of IGF-1 level in serum-free medium of cultured articular chondrocytes (Froger-Gaillard et al., 1989) and was a main autocrine factors in stimulating proteoglycan synthesis, a marker of differentiated chondrocytes, acting through IGF Type 2 receptor (IGF-2R), (Takigawa et al.,1997). The role of IGF-2 in cartilage repairing and cartilage tissue engineering, the relationship between IGF-2 and IGF-1 in these circumstances are still needed to be clarified. Insulin-like growth factor-binding proteins (IGFBPs) represent an evolutionarily conserved protein family including six independent family members: IGFBP1-6. They regulate functions of IGFs and exert functions of storage and transport of IGFs and sometimes IGF-independent actions (Murphy, 1998). Other possible candidates responsible for chondrogenesis include parathyroid hormone-related protein (PTHrP) (Fischer et al., 2010) and extracellular histones (Aung et al., 2011), which have also been found in culture medium of chondrocytes–scaffold constructs and were estimated to be related with chondrogenesis. A further analysis of the soluble factors that are responsible for chondrogenesis and their spatial-temporal sequence would be helpful to develop efficient strategies for chondrogenic differentiation of BMSCs.

In conclusion, the conditioned medium from chondrocyte/scaffold constructs can direct chondrogenic differentiation of BMSCs, and many secreted soluble factors derived from the chondrocytes might play crucial roles in the process.

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