ARTICLE
Journal of Cellular Biochemistry 115:1089–1096 (2014)

Journal of Cellular Biochemistry

Microenvironment Is Involved in Cellular Response to Hydrostatic Pressures During Chondrogenesis of Mesenchymal Stem Cells

Rui Ye,[1] Jin Hao,[1] Jinlin Song,[2] Zhihe Zhao,[1] Shanbao Fang,[1] Yating Wang,[1] and Juan Li[1]*

[1]*State Key Laboratory of Oral Diseases, Department of Orthodontics, West China Hospital of Stomatology, Sichuan University, Chengdu 610041, P.R., China*

[2]*Affiliated Hospital of Stomatology, Chongqing Medical University, Chongqing 404100, P.R., China*

ABSTRACT

Chondrocytes integrate numerous microenvironmental cues to mount physiologically relevant differentiation responses, and the regulation of mechanical signaling in chondrogenic differentiation is now coming into intensive focus. To facilitate tissue-engineered chondrogenesis by mechanical strategy, a thorough understanding about the interactional roles of chemical factors under mechanical stimuli in regulating chondrogenesis is in great need. Therefore, this study attempts to investigate the interaction of rat MSCs with their microenvironment by imposing dynamic and static hydrostatic pressure through modulating gaseous tension above the culture medium. Under dynamic pressure, chemical parameters (pH, pO_2, and pCO_2) were kept in homeostasis. In contrast, pH was remarkably reduced due to increased pCO_2 under static pressure. MSCs under the dynamically pressured microenvironment exhibited a strong accumulation of GAG within and outside the alginate beads, while cells under the statically pressured environment lost newly synthesized GAG into the medium with a speed higher than its production. In addition, the synergic influence on expression of chondrogenic genes was more persistent under dynamic pressure than that under static pressure. This temporal contrast was similar to that of activation of endogenous TGF-β1. Taken altogether, it indicates that a loading strategy which can keep a homeostatic chemical microenvironment is preferred, since it might sustain the stimulatory effects of mechanical stimuli on chondrogenesis via activation of endogenous TGF-β1. J. Cell. Biochem. 115: 1089–1096, 2014.

KEY WORDS: MESENCHYMAL STEM CELLS; CHONDROGENESIS; MICROENVIRONMENT; DYNAMIC HYDROSTATIC PRESSURE; STATIC HYDROSTATIC PRESSURE

The repair of articular cartilage presents great challenges in that cartilage has a limited intrinsic capacity for self-repair. Stem cell based, tissue-engineered strategy has been believed a promising treatment for cartilage repair, and mesenchymal stem cells (MSCs) have been considered as a potential candidate for cell source in clinical applications [Diduch et al., 2000; Ponticiello et al., 2000; Wakitani et al., 2002]. The cartilage in nature is a hydrated connective tissue that withstands and distributes mechanical forces during daily activities. Therefore, further exploration and optimization of mechanical factors affecting MSCs is of great necessity for the sake of successful application of MSCs in cartilage repair.

Microenvironmental factors, such as physical forces and chemical components, are of vital significance for chondrogenic differentiation of MSCs. A large number of studies have elucidated their regulatory impacts, respectively. For example, when hydrostatic pressures within physiological limits were applied to chondrogenically induced MSCs, their differentiation, in terms of chondrocyte-like gene expression [Miyanishi et al., 2006a, b; Finger et al., 2007; Wagner et al., 2008] and cartilage-like ECM accumulation [Miyanishi et al., 2006a; Luo and Seedhom, 2007; Wagner et al., 2008], was promoted. Of equal essence is the chemical component of microenvironment, like oxygen and pH. It has been repeatedly

Grant sponsor: National Natural Science Foundation of China; Grant numbers: 30900286, 81030034; Grant sponsor: Science and Technology Fund of Sichuan Province; Grant number: 2011SZ0096; Grant sponsor: National Natural Science Foundation of China; Grant number: 31370992.

*Correspondence to: Juan Li, DDS, Ph.D., State Key Laboratory of Oral Disease, Department of Orthodontics, West China Hospital of Stomatology, Sichuan University, 14#, 3rd Section, Renmin South Road, Chengdu 610041, P.R. China. E-mail: lijuan@scu.edu.cn

Manuscript Received: 5 December 2013; Manuscript Accepted: 10 December 2013

Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 19 December 2013

DOI 10.1002/jcb.24743 • © 2013 Wiley Periodicals, Inc.

shown that oxygen environment [Obradovic et al., 1999; Scherer et al., 2004; Meyer et al., 2010; Pattappa et al., 2011] and extracellular acidity [Boustany et al., 1995; Razaq et al., 2003] were closely related with chondrogenic differentiation of chondrocyte precursors and behaviors of chondrocytes.

However, previous investigations might have neglected that hydrostatic pressures and those forementioned chemical regulators are sometimes interacted, especially when the force was applied by compressing the gas above the culture medium. It seems that the negligence on the complicated interplay between hydrostatic pressures and chemical microenvironment might cripple our understanding on the genuine mechanobiological responses of MSCs. To bridge the gap of present understanding, our study established a loading system in which the gas environment and extracellular acidity could be supervised. By comparing the effects of dynamic and static pressures which were imposed by changing gaseous tension above the surface of culture, we attempted to preliminarily elucidate the involvement of chemical properties of the microenvironment in influencing chondrogenesis of MSCs.

MATERIALS AND METHODS

CELL CULTURE AND CHONDROGENIC INDUCTION

The bone marrow mesenchymal cells (BMSCs) were harvested from bone marrow of 2-week-old male Sprague–Dawley rats as previously described [Li et al., 2009]. Briefly, the posterior tibias were removed and soft tissues were detached. Metaphysis were resected and bone marrow cells were collected by flushing the diaphysis with a 5-ml syringe. The collected cells were then centrifuged at 200*g* for 10 min to form cell pellet. After removing supernatant, the cell pellet was cultured in α-MEM (Gibco, Grand Island, NY) supplemented with 10% FBS (Gibco). Non-adherent cells were removed away by changing culture medium 48 h later. As the culture reached almost complete confluence, the cells were subcultured at a density of 10,000 cells/cm^2.

The passage two cells were dissociated into 1.5% alginate (Sigma, St. Louis, MO) at a density of 4.0×10^6 cells/ml. Later 120 mM $CaCl_2$ were added into the solution for 10 min to produce alginate beads. After the spherical beads were formed, they were rinsed three times with PBS, and cultured in chondrogenic medium composed of serum-free high-glucose DMEM (Gibco) with the addition of 10 ng/ml recombinant human TGF-β1 (Peprotech, Rocky Hill, NJ), 10^{-7} M dexamethasone (Sigma), 50 mg/ml L-ascorbic acid (Sigma), and 1% ITS-A supplement (Gibco).

DYNAMIC AND STATIC PRESSURE EXPERIMENT

A custom-made, computer-operated loading system was used to deliver dynamic and static compressive pressures to cells. The details of the device were described previously [Nagatomi et al., 2001; Liu et al., 2009; Li et al., 2009, 2012]. Briefly, standard tissue-culture wares with cells were maintained in a sealed chamber, whose pressure was controlled by a computer. Pressure magnitude was modulated by changing gaseous tension in the sealed chamber and the frequence was regulated by the computer-driven motors. When the frequence was set to 0, cells were exposed to static hydrostatic pressure. Two pressure sensors were linked with the pressure chamber to monitor the real-time pressure value in the chamber. During the loading experiment, the sealed chamber was maintained under standard cell culture conditions, that is, 37°C, humidified, 95% air, and 5% CO_2 air environment.

Mechanical stress was initiated at the 8th day of chondrogenic culture, based on the finding of our previous research [Li et al., 2012] that showed rat BMSCs began to exhibit typical chondrocyte features after 7 days′ induction. Cells were exposed to dynamic (14–36 kPa, at 0.25 Hz frequency and with a sinusoidal wave) or static (20 kPa) pressure, respectively (Fig. 1A). A daily regimen was delivered at 1 h per day. The loading experiments were conducted for 1, 3, 5, and 7 consecutive days. Control cells were cultured in the same culture conditions for the same period of time without loading. All the experiments were conducted for three times.

MEDIUM SAMPLING AND MEASUREMENT OF CHEMICAL PARAMETERS

Medium of the specimens was replaced before each loading experiment. As the loading was initiated, every 2 mL of culture medium from each specimen was sampled by a syringe at the following time point: 0 min, 15 min, 30 min, 45 min, and 1 h during loading, as well as 1 and 4 h after loading. Collected medium was immediately analyzed after sampling for pO_2, partial pressure of CO_2, and pH using a blood gas analyzer (Nova Biomedical, Waltham, MA). To determine whether these alteration in parameters is a direct consequence of mechanical loading or an indirect one of loading-induced cell metabolism, culture medium without cells were sampled and analyzed as described before. All the experiments were conducted for three times on every day of loading.

HISTOLOGICAL AND IMMUNOHISTOCHEMICAL ANALYSIS

Alginate beads at indicated time were rinsed with PBS for three times, hardened in 55 mM $CaCl_2$ solution, fixed in 4% paraformaldehyde for 30 min, dehydrated, and embedded in paraffin. Sections (5 μm thick) were stained with 0.2% Toluidine blue and 0.5% Safranin-O to visualize sulfate cartilage matrix proteoglycan. Type II collagen depositions were detected with immunostaining with rabbit polyclonal antibody against type II collagen (Boster Bio-Tech, China, diluted 1:200).

GLYCOSAMINOGLYCAN (GAG) QUANTITATIVE ANALYSIS

The GAG contents in the alginate beads and culture medium were assessed after loading. Wet weights of the alginate beads were measured and frozen at −80°C for later analyses. Samples ($n = 5$ for each group) were incubated overnight at 60°C in a papain solution containing 125 mg/ml papain, 5 mM L-cystein, 5 mM EDTA, and 100 mM sodium phosphate. The GAG contents were estimated by the 1,9-dimethylmethylene blue assay, and chondroitin sulfate (Sigma) was used as a standard for quantification. GAG contents were normalized to wet weight of alginate beads. The GAG value of the 1st day was designated as 1. Results were presented as fold inductions over that of the 1st day.

REAL-TIME RT-PCR ANALYSIS

Cells in the alginate beads were collected and dissolved into Trizol Reagent (Invitrogen, Carlsbad, CA) after mechanical loading. Total mRNA was extracted and reverse-transcribed into cDNA by Mmlv

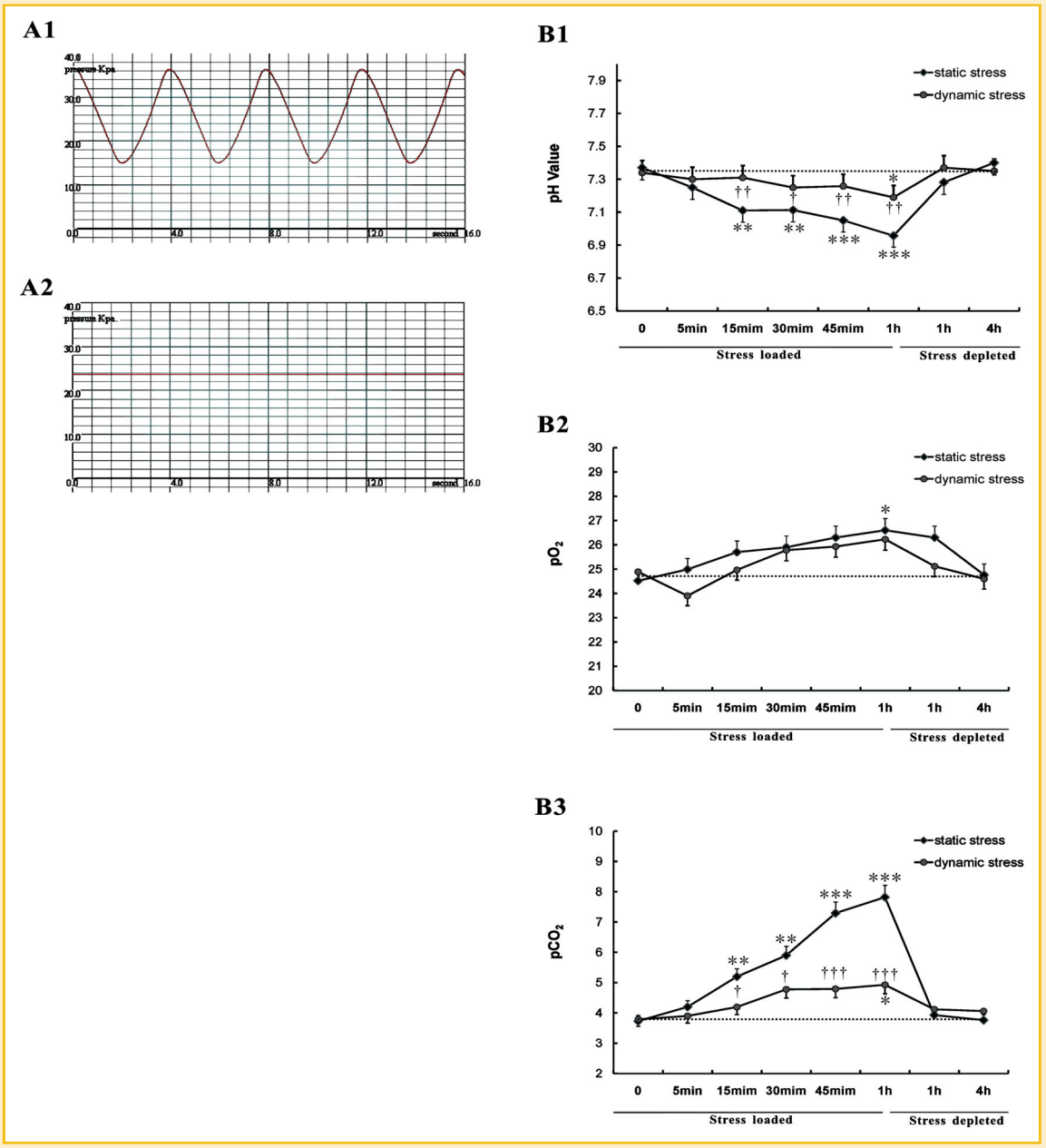

Fig. 1. Influences of dynamic and static hydrostatic pressures on microenvironment of cells cultured in alginate beads. Specimens were cultured in chondrogenic induction medium for 7 days, and then subjected to hydrostatic pressures which were imposed by compressing gas above the culture medium. A: The screenshot of the monitor system when specimens were exposed to sinusoidal dynamic pressure (A1, 0.25 Hz) or static pressure (A2, 0 Hz). B: Changes of culture microenvironment accompanied with the loading process. Culture medium was sampled at the following time point: 0 min, 5 min, 15 min, 30 min, 45 min, and 1 h during loading, as well as 1 and 4 h after loading. Collected medium was analyzed by a blood gas analyzer. The dotted line showed the baseline level, which was equivalent to pre-loading level of the control cells. (B1) pH; (B2) pO_2; (B3) pCO_2. *Significant difference between dynamic/static pressure and unloaded control; †significant difference between dynamic pressure and static pressure. $^{*,\dagger}P < 0.05$, $^{**,\dagger\dagger}P < 0.01$, $^{***,\dagger\dagger\dagger}P < 0.001$.

reverse transcriptase (Invitrogen) according to the manufacturer's protocol. Real-time PCR was carried out using the SYBR Prime Script™ RT-PCR kit (Takara, Dalian, China) in an ABI PRISM 7300 Real-Time PCR System. Col2α1, Aggrecan, and Sox9 were amplified with primer sequences listed in our previous report [Li et al., 2009]: the housekeeping gene, GAPDH, was also amplified as a loading control. The PCR program was initiated by 30 s at 95°C before 40 thermal cycles, each of 5 s at 95°C and 31 s at 60°C. Sterilized ddH_2O was used as generated from a dilution series of an arbitrary sample. Results were normalized to GAPDH. Control value was designated as 1. Results were presented as fold inductions over that of the controls.

ELISA ANALYSIS

The exogenous supplement TGF-β1 was ceased at the 8th day of chondrogenic culture, and static and dynamic stresses were subsequently delivered to the cells. The volume of the culture medium was kept equivalent for each specimen. ELISA analysis was used to detect endogenous TGF-β1 secretion at indicated time points. The concentrations of TGF-β1 in culture supernatants were measured using a commercial TGF-β1 ELISA kit (R&D, USA) following the manufacturer's protocol. The OD value was read by a spectrophotometer at 450 nm. The TGF-β1 production was calculated by the standard curve produced from serial dilutions. Bioactive TGF-β1 was directly detected by the reagent. Latent TGF-β1 was determined after activation with 1 M HCl as the agent's instruction. The unloaded TGF-β1-discontinued specimens were used as controls.

STATISTICAL ANALYSIS

Data of evaluated parameters were presented as mean ± SD, and analyzed by factorial analysis of variance (ANOVA) followed by Newman–Keuls test. $P < 0.05$ was considered statistically significant.

RESULTS

ALTERATION OF MICROENVIRONMENT IN CULTURE MEDIUM

Figure 1B showed the representative changes in chemical properties of the medium accompanied by loading. The dotted line showed the baseline level, which was equivalent to pre-loading level of the control cells. Their pH, pO_2, and pCO_2 stayed stable near the baseline throughout the whole period (data now shown).

The pO_2 was altered in similar way by both dynamic and static pressures, with a slight increase during loading. However, alterations of pH and pCO_2 bore no resemblances between the two pressures. The pH slightly fluctuated under the baseline during dynamic loading, while it dramatically declined under static pressure and hit the bottom by the end of static loading. The pCO_2 gently rose as the dynamic pressure was loaded, but rocketed under static pressure, and peaked at approximately twofold higher than pre-loading level by the end of loading. All the parameters came close to baseline levels within 4 h after loading and stayed stable thereafter. Similar results were obtained if medium was sampled without cell constructs (data not shown). The parameters altered in similar ways on each day of loading.

HISTOLOGICAL ANALYSIS OF ECM DEPOSITION

After 14 days' chondrogenic culture, unloaded control cells exhibited typical round shape of chondrocytes, embedded within Toluidine blue and Safranin-O stained proteoglycan-rich matrix and immunohistochemically stained type II collagen (Fig. 2). As dynamic pressure was applied for 7 days, cells still showed similar phenotypes, but the staining of their extracellular matrix (ECM) and type II collagen tended to be stronger. In contrast, cells exposed to static pressure exhibited less ECM deposition and type II collagen production, with a depth of stain similar to that of control cells.

GAG SYNTHESIS IN RESPONSE TO HYDROSTATIC PRESSURES

GAG gradually accumulated in both the alginate beads and culture medium for unloaded controls (Fig. 3). When the cells were exposed to dynamic compressive stress initiated at the 8th day, GAG secretion

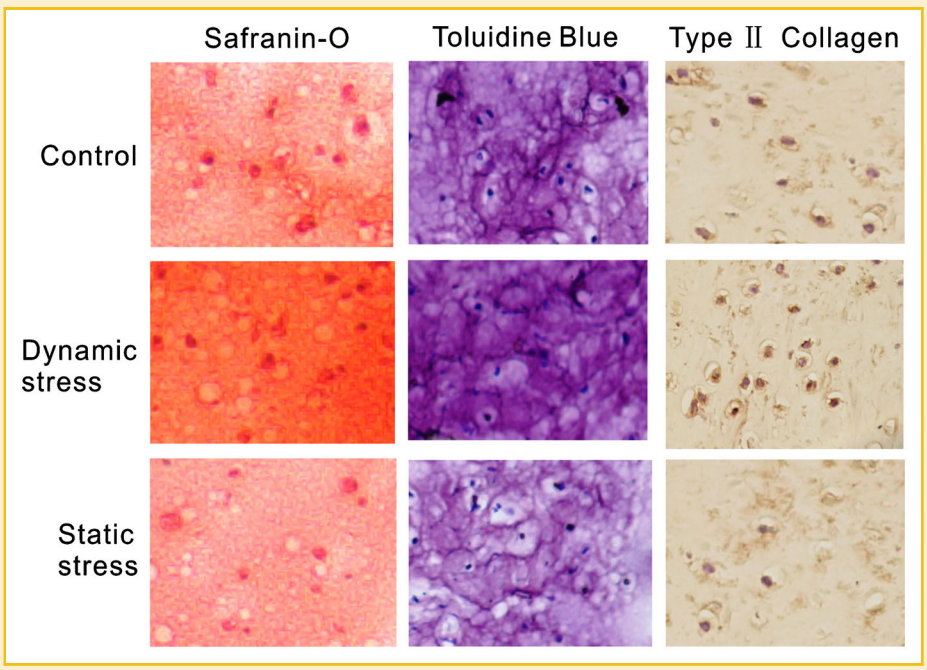

Fig. 2. Phenotypical alterations of chondrogenic BMSCs (×200) stimulated by dynamic and static pressure. Rat BMSCs were cultured in chondrogenic induction medium for 7 days, then subjected to hydrostatic pressures for another 7 days, and examined for Toluidine Blue staining, Safranin-O staining, and type II collagen immunohistochemical analysis at the 14th day.

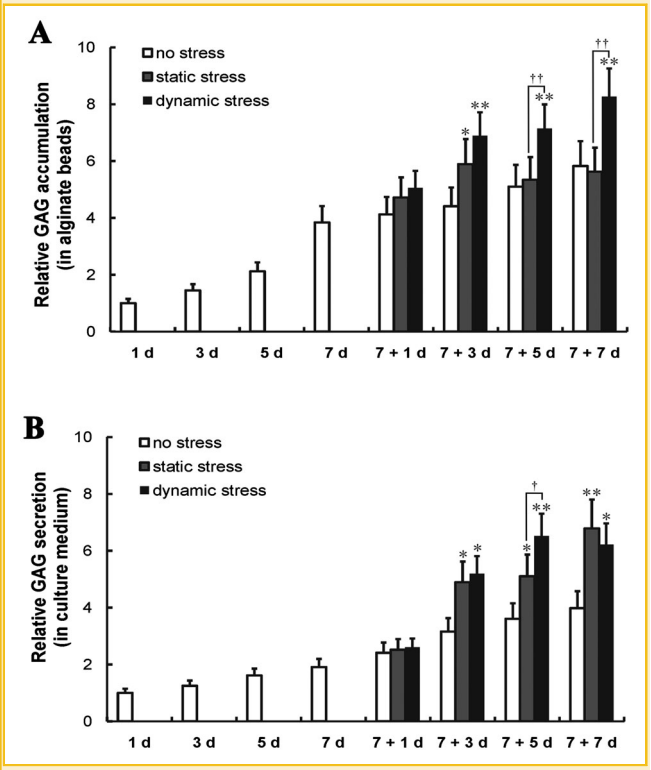

Fig. 3. GAG deposition stimulated by pressures. Specimens were cultured in chondrogenic medium supplemented with TGF-β1 for 7 days, and mechanical stress was applied for 1, 3, 5, and 7 consecutive days. GAG in the alginate beads (A) and culture medium (B) were quantified at indicated time points. Results showed GAG persistently accumulated in the alginate beads and the medium under dynamic pressure, but it was lost to the medium with a speed higher than its production under static pressure. *Significant difference between dynamic/static pressure and unloaded control; †significant difference between dynamic pressure and static pressure. $^{*,\dagger}P < 0.05$, $^{**,\dagger\dagger}P < 0.01$, $^{***,\dagger\dagger\dagger}P < 0.001$.

was boosted within the alginate beads and culture medium, and got significantly higher than the unloaded controls after being loaded for 3, 5, and 7 days. In contrast, as the cells were exposed to static compressive stress, the content of GAG within alginate beads increased for the first 3 days and decreased afterwards. In the meanwhile, GAG in the culture medium was persistently increased.

CHONDROGENEIC GENE EXPRESSION IN RESPONSE TO HYDROSTATIC PRESSURES

CHONDROGENIC GENE TRANSCRIPTION WITH CONTINUOUS SUPPLEMENT OF TGF-β1. The loading experiments showed both types of pressures imposed synergic influences on transcription of chondrogenic genes, but the effect by static pressure was less persistent and less pronounced (Fig. 4A). Based on the gradual increasing trend under no stress condition, dynamic pressure significantly elevated the mRNA levels of Col2α1, Aggrecan, and Sox9 throughout the period of loading experiment. In contrast, static pressure was able to promote their expressions for the first 3 or 5 days with a less magnitude than the one under dynamic pressure.

CHONDROGENIC GENE TRANSCRIPTION WITH DEPLETION OF TGF-β1 AT 8TH DAY. The expression of chondrogenic genes ceased to increase under no stress condition after exogenous TGF-β1 was discontinued at 8th day (Fig. 4B). Dynamic stress significantly promoted the expression of Col2α1, Aggrecan, and Sox9 for at least 5 days. In contrast, the promotion effects by static pressures were transient, usually being evident on the 3rd or 5th day of loading. The mRNA levels of chondrogenic genes tended to slump after the transient rise, some of which even dropped less than the ones of unloaded control by the end of experiments.

ENDOGENOUS TGF-β1 SECRETION

When the exogenous supplement of TGF-β1 was ceased at the 8th day of chondrogenic culture, the concentration of TGF-β1 of control cells gradually declined. Dynamic pressure persistently raised the TGF-β1 levels in both active (Fig. 5A) and total (active plus latent) forms (Fig. 5B) throughout the period of experiments. In contrast, the elevation of TGF-β1 under static stress was less lasting. The concentration of active TGF-β1 peaked on the 3rd day of loading following a similar rise as that under dynamic pressure, and slumped afterwards. For total TGF-β1, it was raised by static stress for 5 days and declined later on.

DISCUSSION

It has been long expected to generate functional cartilage equivalents for potential clinical use by culturing MSCs within chondrogenic microenvironment. The device used by the present study was able to render a chondrogenic microenvironment by imposing various mechanical and chemical factors. Previous studies [Li et al., 2009; Meyer et al., 2010] confirmed that MSCs loaded by the device could sense the mechanical stimuli and consequently undergo promoted chondrogenesis, but these studies overlooked the fact that cells were also subjected to changes of culture medium by the time of loading, which might have also affected the mechanobiological responses. According to our findings, when the stress was implemented by pressurizing the gases above the culture medium [Li et al., 2009, 2012; Liu et al., 2009], concentration of dissolved gases and acidity of the medium was altered by the time of loading. Specifically, static pressure was associated with dramatically reduced pH, which was largely due to accumulation of dissolved CO_2 within the medium. In contrast, dynamic pressure almost kept pH and pCO_2 in homeostasis. This was probably the result of a sinusoidal fluctuation in the magnitude of gaseous tension that applied to the cells, which might enhance the gas exchange between the chamber and the medium, and therefore buffered accumulation of CO_2. Oxygen tension (pO_2) was insignificantly increased under both types of pressures, so the oxygen supply was guaranteed within the system. In our system, altered parameters within the medium were primarily a consequence of loading other than that of cell metabolism, since similar changes occurred when cell constructs were removed from the medium, as well as on each day of loading after replacing the medium with fresh one. It is true that biological responses of MSCs might have influenced the medium condition in the long run, but, in our research, they seemed insufficient to significantly alter the chemical parameters over the few hours as we supervised.

Results obtained from histological staining and GAG quantification showed that static pressure adversely affected histological appearance and ECM synthesis of the cell beads at the end of culture

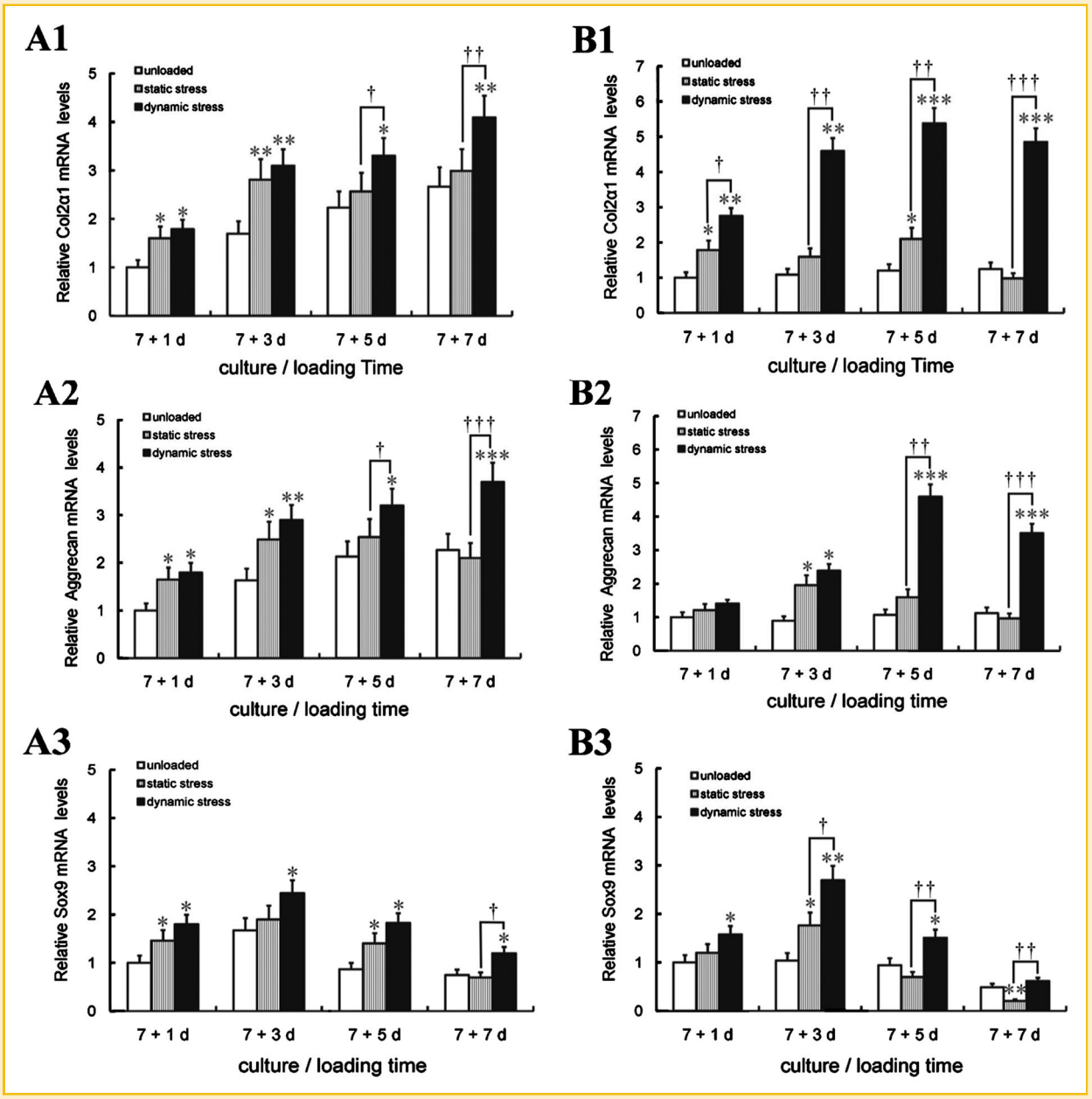

Fig. 4. Gene expression stimulated by pressures. The results showed both dynamic and static pressures promoted transcription of chondrogenic genes, but the effects of static pressure were less persistent and less pronounced. A: Chondrogenic gene expression with continuous supplement of TGF-β1. B: Chondrogenic gene expression when supplement of TGF-β1 was deprived after the 7th day. (A1/B1) Col2α; (A2/B2) Aggrecan; (A3/B3) Sox9. *Significant difference between dynamic/static pressure and unloaded control; †significant difference between dynamic pressure and static pressure. $^{*,\dagger}P < 0.05$, $^{**,\dagger\dagger}P < 0.01$, $^{***,\dagger\dagger\dagger}P < 0.001$.

period under static pressure, as compared to under dynamic pressure. Of note, although increasing GAG deposited within the alginate beads for the first 3 days under static pressure, it was lost into the culture medium with a speed higher than its production. This resulted in a return to control level of GAG deposition within the alginate beads during the later phase of culture (12–14 days). By contrast, GAG continuously accumulated within both the beads and the medium under dynamic pressure, indicating a strongly synergic effect of dynamic pressure on GAG synthesis. Consistent with previous reports [Sah et al., 1991; Lee and Bader, 1997], the application of stress increased release of newly synthesized GAG into the medium, attributed to an increase in the rate of passive diffusion of the macromolecules out of the cell constructs due to stress-induced fluid flow. However, unlike the conclusion drawn from the previous research, although the dynamic pressure resulted in an enhanced fluid flow, it did not induce a greater release. This may be due to acidification of the culture condition by the static pressure. According to another previous investigation, increasing GAG tended to diffuse

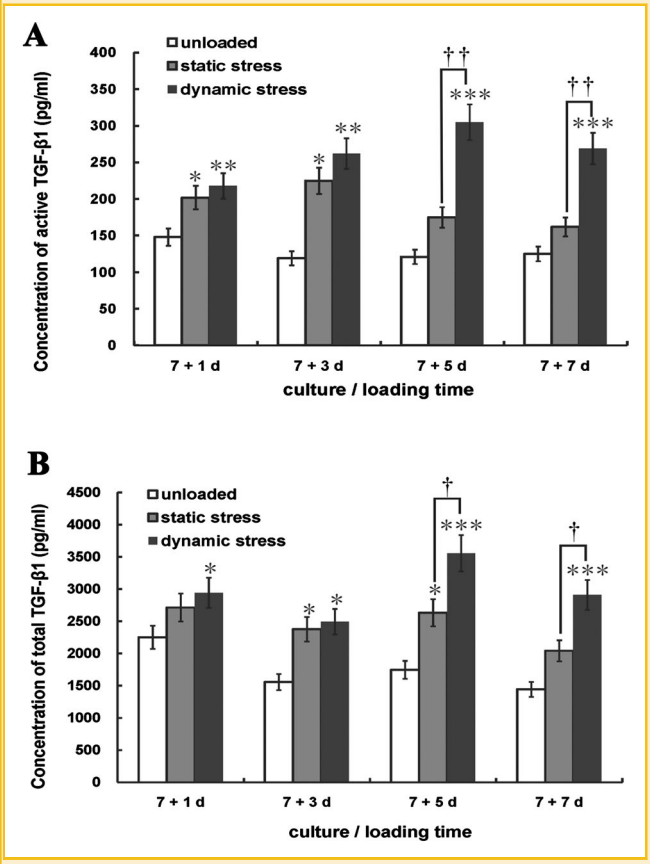

Fig. 5. TGF-β1 protein secretion stimulated by pressures. After 7 days' chondrogenic culture, BMSCs were exposed to chondrogenic defined medium without exogenous supplement of TGF-β1, and then subjected to delayed stress for 1, 3, 5, and 7 days, respectively. The culture medium was collected for ELISA detection. Results showed that both dynamic and static pressures stimulated TGF-β1 protein secretion, but the secretion under dymanic pressure was more prominent and persistent. (A) active TGF-β1; (B) total TGF-β1. *Significant difference between dynamic/static pressure and unloaded control; †significant difference between dynamic pressure and static pressure. $^{*,\dagger}P < 0.05$, $^{**,\dagger\dagger}P < 0.01$, $^{***,\dagger\dagger\dagger}P < 0.001$.

into the medium under acidic environment [Obradovic et al., 1999]. It was conceivable, therefore, that alteration of culture microenvironment accounted for the extra loss of GAG under static pressure.

Results of chondrogenic transcripts showed a less persistent stimulatory effect of static pressure than to that of dynamic pressure. Specifically, gene transcriptions were enhanced for the first 3 or 5 days of loading under static pressure, and this synergic effect diminished in the following days. Similar trend could also be roughly observed in the amount of GAG synthesized, by summing up GAG quantity within and outside the alginate beads. Such temporal difference in chondrogenic promotion by the two types of pressures could be at least partly explained by their different alterations in culture microenvironment. As previously described, MSCs within our system not only sensed the mechanical signals but also the chemical cues derived from changes in microenvironment. It has been validated that pressures within physiological ranges [Li et al., 2012; Li et al., 2009] promoted ECM production and chondrogenic differentiation whereas acidic medium imposed opposite roles [Obradovic et al., 1999]. We thus speculated that it was medium acidification generated by the static pressure that gradually offset and finally overturned the synergic effects of the pressure itself. By contrast, with efficient gas exchange that served to buffer the medium and keep it in homeostasis, chondrogenesis was continuously promoted by the dynamic pressure.

The previous findings indicated that chemical microenvironment might have participated in the regulation of chondrogenesis under mechanical stimuli, but the inherent mechanism remained elusive. TGF-β1 has been considered as the most important growth factor used in chondrogenic induction, and it was recently reported to be involved in cellular response to mechanical stimulation during chondrogenic differentiation [Huang et al., 2004, 2005]. We have previously found that dynamic pressure could compensate the reduction in chondrogenesis caused by withdrawal of TGF-β1 and relay chondrogenic differentiation via activation of endogenous TGF-β1. Consistent with this previous research, we found secretion of endogenous TGF-β1 was persistently activated under dynamic pressure within TGF-β1-free medium. However, the process was transiently activated under static pressure. This temporal comparison in activation of endogenous TGF-β1 resembled that in expression of chondrogenic genes loaded by the two types of pressures within TGF-β1-free medium. These data suggested that activation of endogenous TGF-β might serve as a necessary pathway for culture microenvironment to realize its regulatory role under pressures.

In summary, we took initiative to demonstrate the differences of rat MSCs in response to dynamic and static hydrostatic pressures that led to varying alterations in culture microenvironment. The findings indicated that loading-induced acidification of culture medium might contribute to adverse influences on chondrogenesis. Thus, to keep homeostasis of culture microenvironment by means of efficient gas exchange or buffer system served as one indispensable concern in optimization of mechanical factors affecting MSCs' chondrogenesis.

REFERENCES

Boustany NN, Gray ML, Black AC, Hunziker EB. 1995. Time-dependent changes in the response of cartilage to static compression suggest interstitial pH is not the only signaling mechanism. J Orthop Res 13:740–750.

Diduch DR, Jordan LC, Mierisch CM, Balian G. 2000. Marrow stromal cells embedded in alginate for repair of osteochondral defects. Arthroscopy 16:571–577.

Finger AR, Sargent CY, Dulaney KO, Bernacki SH, Loboa EG. 2007. Differential effects on messenger ribonucleic acid expression by bone marrow-derived human mesenchymal stem cells seeded in agarose constructs due to ramped and steady applications of cyclic hydrostatic pressure. Tissue Eng 13:1151–1158.

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.

Huang CY, Reuben PM, Cheung HS. 2005. Temporal expression patterns and corresponding protein inductions of early responsive genes in rabbit bone marrow-derived mesenchymal stem cells under cyclic compressive loading. Stem Cells 23:1113–1121.

Lee DA, Bader DL. 1997. Compressive strains at physiological frequencies influence the metabolism of chondrocytes seeded in agarose. J Orthop Res 15:181–188.

Li J, Zhao Z, Yang J, Liu J, Wang J, Li X, Liu Y. 2009. p38 MAPK mediated in compressive stress-induced chondrogenesis of rat bone marrow MSCs in 3D alginate scaffolds. J Cell Physiol 221:609–617.

Li J, Wang J, Zou Y, Zhang Y, Long D, Lei L, Tan L, Ye R, Wang X, Zhao Z. 2012. The influence of delayed compressive stress on TGF-beta1-induced chondrogenic differentiation of rat BMSCs through Smad-dependent and Smad-independent pathways. Biomaterials 33:8395–8405.

Liu J, Zhao Z, Li J, Zou L, Shuler C, Zou Y, Huang X, Li M, Wang J. 2009. Hydrostatic pressures promote initial osteodifferentiation with ERK1/2 not p38 MAPK signaling involved. J Cell Biochem 107:224–232.

Luo ZJ, Seedhom BB. 2007. Light and low-frequency pulsatile hydrostatic pressure enhances extracellular matrix formation by bone marrow mesenchymal cells: An in-vitro study with special reference to cartilage repair. Proc Inst Mech Eng H 221:499–507.

Meyer EGBC, Thorpe SD, Kelly DJ. 2010. Low oxygen tension is a more potent promoter of chondrogenic differentiation than dynamic compression. J Biomech 43:2516–2523.

Miyanishi K, Trindade MC, Lindsey DP, Beaupre GS, Carter DR, Goodman SB, Schurman DJ, Smith RL. 2006a. Dose- and time-dependent effects of cyclic hydrostatic pressure on transforming growth factor-beta3-induced chondrogenesis by adult human mesenchymal stem cells in vitro. Tissue Eng 12:2253–2262.

Miyanishi K, Trindade MC, Lindsey DP, Beaupre GS, Carter DR, Goodman SB, Schurman DJ, Smith RL. 2006b. Effects of hydrostatic pressure and transforming growth factor-beta 3 on adult human mesenchymal stem cell chondrogenesis in vitro. Tissue Eng 12:1419–1428.

Nagatomi J, Arulanandam BP, Metzger DW, Meunier A, Bizios R. 2001. Frequency- and duration-dependent effects of cyclic pressure on select bone cell functions. Tissue Eng 7:717–728.

Obradovic B, Carrier RL, Vunjak-Novakovic G, Freed LE. 1999. Gas exchange is essential for bioreactor cultivation of tissue engineered cartilage. Biotechnol Bioeng 63:197–205.

Pattappa G, Heywood HK, de Bruijn JD, Lee DA. 2011. The metabolism of human mesenchymal stem cells during proliferation and differentiation. J Cell Physiol 226:2562–2570.

Ponticiello MSSR, Kadiyala S, Barry FP. 2000. Gelatin-based resorbable sponge as a carrier matrix for human mesenchymal stem cells in cartilage regeneration therapy. J Biomed Mater Res 52:246–255.

Razaq S, Wilkins RJ, Urban JP. 2003. The effect of extracellular pH on matrix turnover by cells of the bovine nucleus pulposus. Eur Spine J 12:341–349.

Sah RL, Doong JY, Grodzinsky AJ, Plaas AH, Sandy JD. 1991. Effects of compression on the loss of newly synthesized proteoglycans and proteins from cartilage explants. Arch Biochem Biophys 286:20–29.

Scherer K, Schunke M, Sellckau R, Hassenpflug J, Kurz B. 2004. The influence of oxygen and hydrostatic pressure on articular chondrocytes and adherent bone marrow cells in vitro. Biorheology 41:323–333.

Wagner DR, Lindsey DP, Li KW, Tummala P, Chandran SE, Smith RL, Longaker MT, Carter DR, Beaupre GS. 2008. Hydrostatic pressure enhances chondrogenic differentiation of human bone marrow stromal cells in osteochondrogenic medium. Ann Biomed Eng 36:813–820.

Wakitani S, Imoto K, Yamamoto T, Saito M, Murata N, Yoneda M. 2002. Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees. Osteoarthritis Cartilage 10:199–206.