Effects of insulin-like growth factor 1 and basic fibroblast growth factor on the morphology and proliferation of chondrocytes embedded in Matrigel in a microfluidic platform

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Abstract. An integrated microfluidic device was utilized in the present study to investigate the morphology and proliferation of rabbit articular chondrocytes embedded in Matrigel in the presence of insulin-like growth factor 1 (IGF-1) and/or basic fibroblast growth factor (bFGF). The microfluidic device was composed of two parallel channels and a central perfusion-based three-dimensional cell culture module. The rabbit chondrocytes were cultured for 2 weeks at series of concentration gradients of IGF-1 and/or bFGF, which were generated through a diffusion process. At the end of the experiment, the morphology and quantity of cells were measured. Since high expression of collagen II is essential to the function of hyaline cartilage, immunofluorescent images of collagen II expression prior to and after the experiments were gathered for each group. The mean fluorescence intensity ratio (MIR) of collagen II in each group was calculated. The MIRs of collagen II in chondrocytes treated with IGF-1 ranged from 0.6-0.81, those in the cells treated with bFGF ranged from 0.47-0.52, and those in cells treated with a combination of IGF-1 and bFGF ranged from 0.63-0.83. Chondrocyte aggregations were observed in the group treated with 75-100 ng/ml IGF-1 (3.46-fold proliferation ratio). Similarly, a 3.83-fold proliferation ratio was identified in chondrocytes treated with 2.5-5.0 ng/ml bFGF. The group treated with 50-75 ng/ml IGF-1 and 2.5-5.0 ng/ml bFGF exhibited the optimum increase in proliferation (4.83-fold proliferation ratio). The microfluidic device used in the present study can be easily adapted to investigate other growth factors at any concentration gradient. In addition, parallel experiments can be performed simultaneously with a small quantity of cells, making it an attractive platform for the high-throughput screening of cell culture parameters. This platform will aid in the optimization of culture conditions for the *in vitro* expansion of chondrocytes while maintaining their *in vivo* morphology, which will improve autologous chondrocyte implantation capabilities for the treatment of cartilage injury.

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Introduction

The capacity of articular cartilage for spontaneous repair is limited due to its unique physiological properties (1). As a consequence, cartilage damage is unable to be repaired, leading to osteoarthritis (2). There is an increasing incidence of cartilage degeneration due to aging and more active populations; however, there remains no cure (3). Numerous non-biological therapeutic interventions for cartilage damage exist, including lavage, debridement, microfracture and abrasion chondroplasty (4-7). Unfortunately, the long-term therapeutic efficacy of these methods is controversial (8). Regenerative medicine using autologous chondrocyte implantation (ACI) is currently of great interest for the treatment of cartilage damage because it is less invasive and is biocompatible *in vivo* for longer periods of time compared with the therapies that are currently available (9). In ACI, a biopsy of the cartilage is taken from the patient's knee by an arthroscopic surgery. Subsequently, the chondrocytes that have been expanded and harvested *in vitro* are directly transplanted back into the defector utilized to engineer implantable grafts (10). However, the use of ACI treatment is limited due to the inevitable dedifferentiation of seeded cells that happens during the *in vitro* expansion (11).

Chondrocytes are typically embedded in a three-dimensional (3D) extracellular matrix for *in vitro* culture. The physical, chemical and biological regulation of the cellular microenvironment is essential to maintain the spatial and temporal behavior of chondrocytes. Cartilage is avascular and aneural; thus, chondrocytes require nourishment by the slow diffusion of synovial fluid (12). Although the traditional two-dimensional (2D) cell culturing technique was typically adopted to explore the optimal expansion environment for chondrocytes *in vitro*, it remains controversial whether chondrocytes grown *in vitro* are morphologically identical to those present *in vivo* (13). The microfluidic platform has been demonstrated to improve various *in vitro* cell cultures. By integrating the dynamics that imitate the human metabolic system, microfluidic devices can conveniently supply and transfer

media, buffers and gas towards the cells cultured inside the device, while the waste products generated from cellular activities are drained (14,15). The microfluidic platform also creates novel abilities for the spatial and temporal regulation of cell growth (16). Therefore, the microfluidic platform may supply a better simulation of the *in vivo* microenvironment compared with conventional static cultures, aiding in the maintenance of the *in vivo* morphology of cells during *in vitro* expansion. Furthermore, the capability of miniaturization of microfluidic devices reduces the number of cells required and allows parallel experiments to be performed simultaneously, which makes microfluidics an attractive high-throughput platform for the screening and expansion of chondrocytes for ACI.

The present study developed a perfusion-based polydimethylsiloxane (PDMS) micro 3D cell culture platform capable of producing a consistent concentration gradient through rapid prototyping. Rabbit articular chondrocytes were cultured for 2 weeks with different concentrations of growth factors that were generated using the platform's diffusion process. The effects of insulin-like growth factor 1 (IGF-1) and basic fibroblast growth factor (bFGF) on the morphology and proliferation of the chondrocytes was investigated. This innovative microfluidic platform containing parallel units of cell-loaded microbioreactors capable of forming consistent gradients of growth factors allows for the high-throughput screening and expansion of chondrocytes, which may aid in the widespread use of ACI for the treatment of cartilage injury.

Materials and methods

Microfluidic device fabrication and design. The microfluidic bioreactor was fabricated using PDMS using rapid prototyping, as described previously (17). Firstly, a design for a device was produced in a computer-aided design (AutoCAD 2007, Autodesk, San Rafael, CA, USA) program. A photomask was then generated using a high-resolution printer according to the CAD file. The mask was utilized for contact photolithography using an SU-8 2075 negative photoresist (MicroChem Corporation, Newton, MA, USA) to produce a negative master mold, consisting of patterned photoresist on a silicon wafer. Positive replicas with embossed channels were fabricated by curing PDMS against the master mold. The access holes and reservoirs with the diameter of 1 mm were punched out of the cured layer using a borer. The PDMS replica contained three of the four walls needed for intact channels. The surface of the PDMS replica and a glass substrate were activated by plasma and then attached immediately. An irreversible sealing between the PDMS and the glass substrate was formed after attaching them manually, which provided the fourth wall of microfluidic channels. Plastic tubes with a larger outer diameter compared with the inner diameter of the port were inserted into the holes. Subsequently, the opposite ends of the tubes were connected to a syringe pump (Orion Sage M362; Orion Corporation, Espoo, Finland) located outside of the incubator to complete the system.

The microfluidic device in the present study, illustrated in Fig. 1, was composed of two parallel channels surrounding a central perfusion-based 3D cell culture module. Three cell culture chambers with the same function were integrated with the parallel channels on either side. The chambers were miniaturized to 2 cm in length, 500 μm in width and 100 μm in height. The volume of each chamber was a ~0.4 μl. The chondrocyte-Matrigel (BD Biosciences, San Jose, CA, USA) mixture could be seeded into the chambers through an input hole and channel. The syringe pump was used to drive fluid flow. Culture media with and without growth factors was infused into the two parallel channels simultaneously. Concentration gradients were gradually achieved across the chondrocyte-Matrigel mixture in the cell culture chambers by continuous perfusion. The device was then kept in an incubator at 37˚C with 5% CO_2 and 100% relative humidity. The flow speed was controlled at a rate of 0.1 μl/min.

Chondrocyte isolation and expansion. Full-thickness articular cartilage was harvested from the femoral heads of an adult New Zealand rabbit (4 months old; weight, 2 kg; supplied by Dalian Medical University, Dalian, China; living conditions, 23˚C, 12-h light/dark cycle, free access to food and water) and subsequently minced into 1 mm^3 pieces. The animal work in the present study was approved by the Ethics Committee of Dalian Medical University. The cartilage fragments were then washed twice with PBS for 2 min each time and subjected to collagenase II (0.25% w/v; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) digestion for 10 h at 37˚C. Chondrocytes released from the cartilage were then cultured in a 2D culture system with Dulbecco's modified Eagle's medium supplemented with 20% fetal calf serum (both Sigma-Aldrich; Merck KGaA), 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco™; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Cells were incubated in a humidified incubator at 37˚C with 5% CO_2 and 100% relative humidity. The medium was replaced every 3 days. When the primary chondrocytes reached ~80% confluency, they were harvested using 0.25% trypsin/1.0 mM EDTA (Sigma-Aldrich; Merck KGaA).

Concentration gradient. To characterize the diffusion of IGF-1 and bFGF in Matrigel in the microfluidic bioreactors, fluorescein isothiocyanate (FITC)-dextran (Sigma-Aldrich; Merck KGaA) with a molecular weight of 20 kDa (similar to that of IGF-1 and bFGF) was added to the medium and infused through one side of the fluid channel, while the other fluid channel was infused with medium without FITC-dextran. The infusion rate was maintained at a rate of 0.1 μl/min. Time-lapse fluorescence images of the Matrigel in the bioreactor chamber were taken every minute.

Chondrocyte seeding, culture and assaying. When the chondrocytes reached the plateau growth phase at the end of 2D culturing, they were harvested as described above and embedded in Matrigel solution (1 mg/ml) at a density of $2x10^5$ cells/ml at 4˚C. The chondrocyte-Matrigel mixture was loaded into the culture chambers of the microfluidic chips by pipetting ~0.4 μl/chamber. IGF-1 (100 ng/ml) and bFGF (10 ng/ml) (both PeproTech, Inc., Rocky Hill, NJ, USA) were prepared with the serum-free medium (DMEM with 100 U/ml penicillin and 100 μg/ml streptomycin). From the two inlets the following solutions were infused into the three sets of microfluidic devices, respectively: i) IGF-1; ii) bFGF; and iii) IGF-1 and bFGF. Chondrocytes were cultured at a series of concentrations of IGF-1 (0-25, 25-50, 50-75 and 75-100 ng/ml)

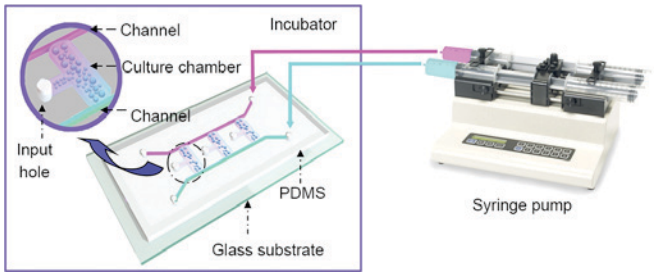

Figure 1. Illustration of the microfluidic device. The microfluidic device was composed of two parallel channels and a central perfusion-based three-dimensional cell culture module. The chondrocytes-Matrigel mixture could be seeded into the chambers through an input hole and channel. A syringe pump was used to drive flow of fluid. Culture media with and without growth factors was infused into the two parallel channels simultaneously. Concentration gradients were gradually achieved across the chondrocyte-Matrigel mixture in the cell culture chambers by continuous perfusion. PDMS, polydimethylsiloxane.

and/or bFGF (0-2.5, 2.5-5, 5-7.5 and 7.5-10 ng/ml) on the microfluidic chips for 2 weeks.

The cells were immunostained for collagen type II, as described in a previous study (18), prior to and after the experiments. Immunofluorescent images were gathered for each group using confocal laser scanning microscopy (LSM 710; Carl Zeiss AG, Oberkochen, Germany). The data were analyzed using ZEN 2010 (Carl Zeiss AG) and Image J (National Institutes of Health, Bethesda, MD, USA) software. The mean fluorescence intensity ratio (MIR) of collagen II in each group was calculated using the software described above. Since PDMS and glass are optically transparent, the number of chondrocytes on the chips could be determined manually with an inverted light microscope prior to and after the experiments, in order to calculate the mean proliferation ratio.

Statistical analysis. Results are presented as the mean ± standard error of the mean from three independent experiments. Pearson's correlation coefficient was used to test the concentration gradient performance. Two-way analysis of variance and a post hoc Fisher's least significant difference test were used to compare groups. Statistical tests were performed using SPSS 14.0 (SPSS, Inc., Chicago, IL, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Consistent concentration gradient of IGF-1 and bFGF in the microfluidic device. To characterize the diffusionof IGF-1 and bFGF within the Matrigel in the microfluidic bioreactors, FITC-dextran with a molecular weight similar to IGF-1 and bFGF was added to the medium through one fluid channel, while the other fluid channel was infused with medium without FITC-dextran. The fluorescent time-lapse images demonstrated the gradual diffusion of FITC-dextran into the Matrigel, with a stable gradient achieved after ~20 min of perfusion (Fig. 2A). Quantification of the fluorescent time-lapse images confirmed that there was a linear steady-state diffusion gradient for FITC-dextran in the microbioreactors (Fig. 2B). The concentration of FITC-dextran at different locations in the chamber could be calculated from the calibration curve (Fig. 2B).

Chondrocyte morphology in the microfluidic culture platform. The biological characteristics of chondrocytes were investigated by comparing their morphologies in 2D culture and in the 3D culture of the microfluidic bioreactors. The cells exhibited fibroblast-like morphology with a spindle-shaped appearance whilst in 2D culture (Fig. 3A). However, chondrocytes embedded in Matrigel in the microfluidic device maintained their spherical-shaped morphology after 2 weeks of culture (Fig. 3B). Immunofluorescent images of collagen II were captured at the end of 2D culturing (Fig. 3C) and 2 weeks after 3D culturing (Fig. 3D-F).

Collagen II MIR increased in chondrocytes treated with IGF-1 and bFGF in the microfluidic culture platform in a concentration-dependent manner. The MIR of collagen II in the chondrocytes treated with different concentrations of IGF-1 ranged from 0.6-0.81 (Fig. 4A). The MIRs of collagen II in cells treated with higher concentrations of IGF-1 (≥25 ng/ml) ranged from 0.76-0.81, which were all significantly higher compared with the MIR in cells treated with the lowest concentration of IGF-1 (all $P<0.05$; Fig. 4A). The MIRs of collagen II in chondrocytes treated with different concentrations of bFGF ranged from 0.47-0.52, with no statistical significance between groups (Fig. 4B). The MIRs of collagen II in the cells treated with IGF-1 and bFGF ranged from 0.79-0.83 (Fig. 4C). The MIR for collagen II was significantly lower in the group treated with 0-25 ng/ml IGF-1 and 7.5-10 ng/ml bFGF compared with all other combinations (all $P<0.05$; Fig. 4C).

Chondrocyte proliferation in the microfluidic culture platform. Aggregation of rabbit articular chondrocytes was observed in the area of 75-100 ng/ml of IGF-1 (Fig. 5A). The chondrocyte proliferation ratio of peaked when treated with 75-100 ng/ml IGF at 3.46-fold, which was significantly higher compared with the cells treated with lower concentrations of IGF-1 (all $P<0.01$; Fig. 5A). When treated with bFGF, the cell proliferation ratio peaked at a concentration of 2.5-5.0 ng/ml at 3.83-fold, which was significantly higher compared with the cells treated with other concentrations of bFGF (all $P<0.01$; Fig. 5B). The combination of IGF-1 and bFGF was demonstrated to synergistically promote the proliferation of chondrocytes in the microfluidic device. The group treated with50-75 ng/ml IGF-1 and 2.5-5.0 ng/ml bFGF exhibited the highest increase in the proliferation at 4.83-fold, which was significantly higher compared with the group treated with other combinations (all $P<0.01$; Fig. 5C).

Discussion

Microfluidics is the science of the design and construction of miniaturized devices containing chambers and tunnels through which fluids flows in a controlled manner, which can be used to construct a variety of device, including labs-on-a-chip and inkjet printer heads. Microfluidics brings the benefits of integration, miniaturization and automation to numerous research areas (19).

Various growth factors and/or cytokines contribute to the maintenance of chondrocyte morphology *in vivo*. To mimic the chondrocyte microenvironment *in vivo*, researchers have explored the effects of different combinations and

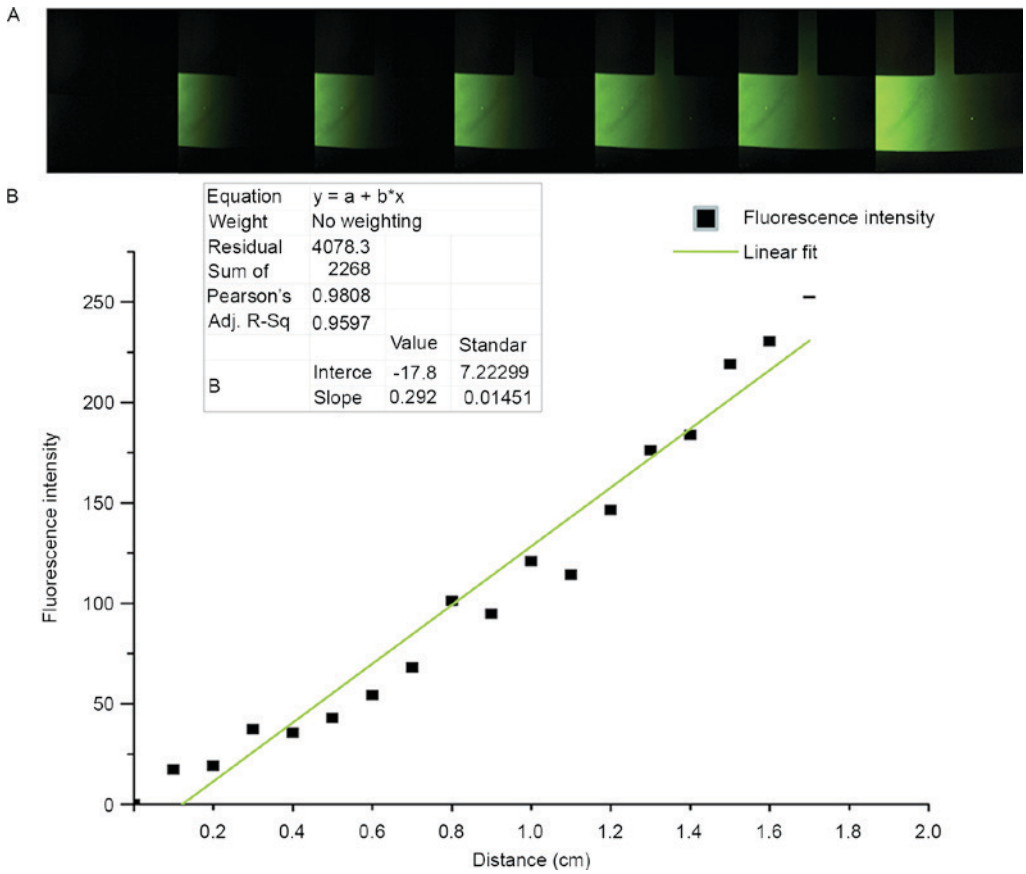

Figure 2. Concentration gradient performance validation. (A) Time-lapse fluorescence images depict the gradual diffusion of FITC-dextran into the Matrigel, with a stable gradient was achieved after ~20 min of perfusion. (B) Quantification of the linear steady-state gradient for FITC-dextran in the microbioreactors, as measured by the fluorescence intensity across the length of the chamber. FITC, fluorescein isothiocyanate.

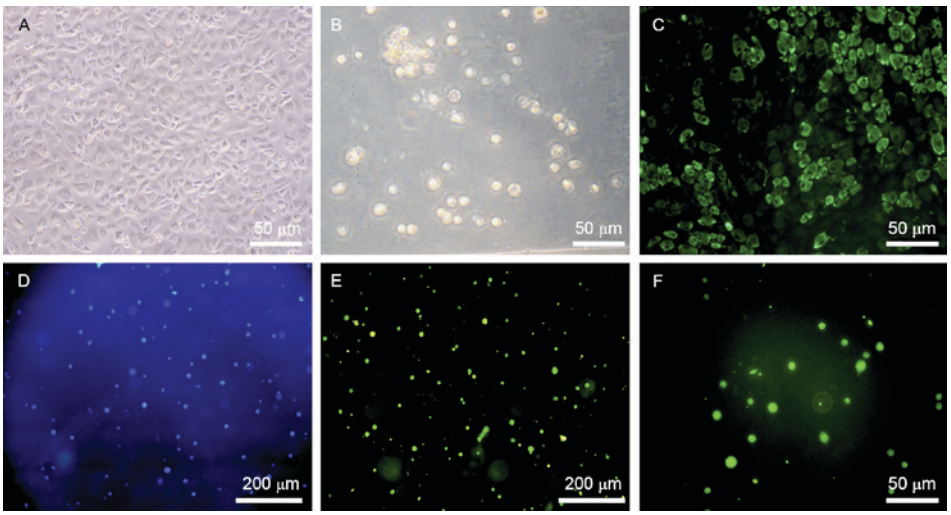

Figure 3. Chondrocyte morphologyin the microfluidic 3D culture platform. (A) The cells exhibited fibroblast-like morphology with a spindle-shaped appearance in 2D culture. (B) Chondrocytes were spherical-shaped in 3D culture. (C) Immunofluorescent image of collagen II in chondrocytes at the end of 2D culturing. (D) Nuclear (4',6-diamidino-2-phenylindole) staining of the chondrocytes at the end of the 3D culture. (E and F) Representative immunofluorescent images of collagen II in the chondrocytes after 2 weeks of 3D culture and treatment with insulin-like growth factor 1 and basic fibroblast growth factor. 2D, two-dimensional; 3D, three-dimensional.

concentrations growth factors on the expansion of chondrocytes *in vitro* (20,21). Unfortunately, few variables could be controlled in previous studies using conventional 2D culture methods. The present study used a microfluidic device, which allowed growth factor concentration gradients to be precisely and automatically controlled across a chondrocyte-Matrigel mixture by continuous perfusion. Constant perfusion maintains the linear boundary conditions of the Matrigel and thus maintains a consistent concentration gradient. Theoretically, using this method the concentration of growth factors in any

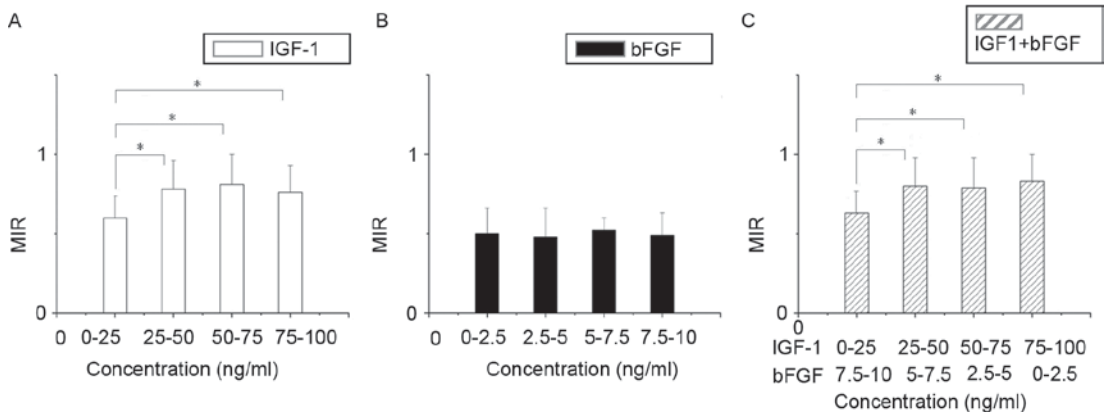

Figure 4. Effect of IGF-1 and/or bFGF on the expression of collagen II by chondrocytes in the microfluidic culture platform. MIR of collagen II in chondrocytes treated with (A) IGF-1, (B) bFGF or (C) IGF-1 and bFGF. *P<0.05 as indicated. IGF-1, insulin-like growth factor 1; bFGF, basic fibroblast growth factor; MIR, mean fluorescence intensity ratio.

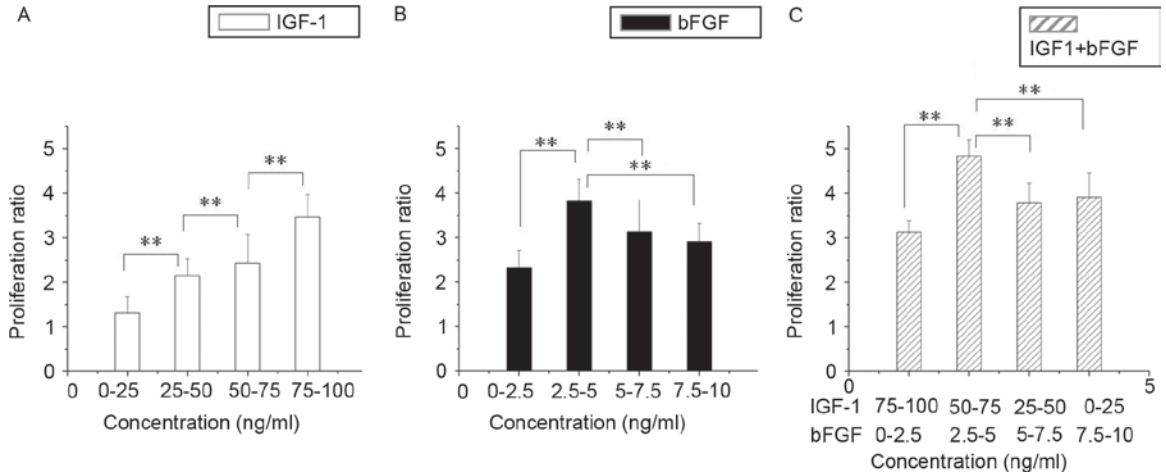

Figure 5. Effect of IGF-1 and/or bFGF on the proliferation of chondrocytes in the microfluidic culture platform. Proliferation ratio of chondrocytes treated with (A) IGF-1, (B) bFGF or (C) IGF-1 and bFGF. **P<0.01 as indicated. IGF-1, insulin-like growth factor 1; bFGF, basic fibroblast growth factor.

area of the device could be calculated using a calibration curve. Furthermore, experimental conditions in the microfluidic device are flexible and can be optimized by changing either the type of input or the concentration of the input. This microfluidic device allows for high-throughput concentration screening that is more convenient and efficient compared with traditional methods. In addition, compared with the concentration gradient generator adopted in our previous studies (22,23) the concentration gradient created using this device is easier to obtain and less susceptible to the external variables.

Culture medium is supplied in a discontinuous manner using traditional experimental set-ups. Cells are cultured under unstable conditions because the medium is changed intermittently and manually, which results in fluctuations in the culture environment and the risk of contamination (15). In the present study, medium supplying nutrients was diffused into the chondrocyte-Matrigel mixture via the lateral channels of the microfluidic device. Meanwhile, cellular metabolic waste was drained. This perfusion system simulated the pattern of cell metabolism *in vivo* and kept the culturing conditions consistent throughout the experimental process. Furthermore, this closed and automated system reduced the possibility of contamination and variation due to manual adjustments.

The optimal *in vitro* culture conditions for chondrocytes are dependent on a variety of factors, including their origin and the type of disease. In ACI, the amount of chondrocytes acquired for expansion *in vitro* is limited. Thus, it is not realistic for researchers to optimize the culture environment using traditional methods, for which the quantity of cells required is typically >10^5-10^6. Using the microfluidic device, parallel experiments could be simultaneously performed using very few cells (~100 cells/experiment). Furthermore, the amount of the medium and growth factors consumed with the microfluidic device used in the present study was <5 ml and <300 ng, respectively. These results demonstrate the advantages of miniaturization, which reduce the consumption of cells and reagents, making microfluidics an attractive platform for high-throughput screening at a cellular level for ACI.

In future, the integrative characteristics of microfluidics could be used to employ more components to manipulate cell behavior, including biomechanical force and hypoxia, into functional devices. This will aid in the identification of specific combinations of elements to optimize the expansion of chondrocytes while maintaining their *in vivo* morphology. This combination should be individual and meet the requirements

of expansion in different phases of growth *in vitro*. If this can be done then it will improve ACI treatment capability.

The maintenance of chondrocyte quality during *in intro* expansion is vital to regenerative medicine for cartilage injury. The high expression of collagen II is important for the function of hyaline cartilage. Thus, the influence of IGF-1 and/or bFGF on the expression of collagen II by chondrocytes in the microfluidic device was investigated in the present study. A previous study demonstrated that 3D culturing is efficient for maintaining the expression of chondrogenic markers and reducing the expression levels of dedifferentiation markers in passaged chondrocytes (24). In the present study, chondrocytes exhibited fibroblast-like morphology with a spindle-shaped appearance on a 2D surface, while those in 3D culture were spherical-shaped, similar to those found in the body.

IGF-1 is a peptide with insulin-like activity that is important for the maintenance of cartilage integrity and extracellular matrix synthesis. In certain studies IGF-1 was demonstrated not to affect collagen deposition (25), while in other studies it caused an increase in collagen synthesis (26). In the present study, the MIR of collagen II in chondrocytes treated with 0-25 and 25-50 ng/ml IGF-1 was ~0.6 and ~0.76, respectively, which did not increase further with increasing IGF-1 concentration. This result suggests that higher concentrations of IGF-1 have little effect on the expression of collagen II in chondrocytes. Mullen *et al* (27) reported higher levels of IGF binding proteins with increasing concentrations of IGF-1, which may competitively bind to and inhibit IGF-1 from binding to its receptor, leading to the hyporesponsiveness of collagen II expression in chondrocytes.

bFGF is synthesized and secreted by human adipocytes. In preliminary animal studies, bFGF has been demonstrated to promote the proliferation of and glycosaminoglycan synthesis by chondrocytes in monolayer (28,29). Another study identified that bFGF enhanced chondrocyte proliferation and promoted the expression of collagen II (30). In the present study, the MIR of collagen II in cells treated with bFGF was maintained at a low level. This result is consistent with a previous investigation (31) that demonstrated that proteoglycan deposition was increased and collagen expression was decreased when chondrocytes were cultured in alginate disks with the addition of bFGF.

The MIRs for collagen II in chondrocytes treated with IGF-1 were higher compared with those treated with bFGF, which indicates that the use of IGF-1 in the maintenance of chondrocyte morphology is more effective compared with bFGF. Collagen II expression in chondrocytes treated with a combination of IGF-1 and bFGF was similar compared with that in chondrocytes treated with IGF-1 alone. These results suggest that any effect on collagen II expression by a combination of IGF-1 and bFGF can be attributed to the effects of 3D culturing and IGF-1 rather than bFGF.

A previous study demonstrated that although the expansion of chondrocytes in 3D culture effectively maintained the chondrocyte phenotype, this method produced a much slower proliferation rate compared with the monolayer culture (32). Thus, it is necessary to accelerate the proliferation of chondrocytes in 3D culture. IGF-I is a major serum-based mitogen for chondrocytes, which has been demonstrated to independently enhance the proliferation of articular chondrocytes (33). Intracellular RAC-alpha serine/threonine-protein kinase (AKT) signaling is initiated when IGF-1 binds to its receptor. The AKT signaling pathway is a stimulator of cell growth and proliferation, and a potent inhibitor of programmed cell death (34). In the present study, ≤100 ng/ml IGF-1 promoted the proliferation of cultured articular chondrocytes in a dose-dependent manner. This is similar to the results of a previous study, which identified that ≤50 ng/ml IGF-I promoted the proliferation of cultured articular chondrocytes in a dose-dependent manner (35). No limitation to proliferation was identified at higher IGF-I concentrations in a previous study (36) or in the present study. In the current study, the proliferation of chondrocytes peaked following treatment with bFGF at a concentration of 2.5-5.0 ng/ml. The proliferation ratio decreased with higher bFGF concentrations. This inhibition may be associated with the changes to extracellular signaling mediated by bFGF (37). However, further studies are required to investigate this effect.

In the current study, a combination of IGF-1 and bFGF was identified to promote chondrocyte proliferation synergistically. The group treated with50-75 ng/ml IGF-1 and 2.5-5.0 ng/ml bFGF exhibited the optimum increase in proliferation. Previous studies revealed inconsistent results regarding the interaction between IGF-1 and bFGF on chondrocyte proliferation. A synergistic effect of FGF-2 and IGF-1 has been proved (38) and disproved (29) in previous studies. This difference may result from differences in a variety of factors, including the species used to obtain chondrocytes, the age of the donor animals, the initial seeding density, the method of cultivation (2D or 3D) and the duration of culture.

Although the chondrocytes could migrate by chemotaxis due to the concentration gradient of IGF-1 and bFGF, the limited displacement reduces the influence of this on the results. Articular cartilage lesions that do not penetrate the underlying subchondral bone fail to heal (39). This failure is thought to be partially due to limited migration to the defect. Nadzir *et al* (40) reported that chondrocyte migration into collagen gel was suppressed by IGF-1 due to the dense collagen gel fibrils surrounding the cells and the downregulation of membrane type 1-matrix metalloproteinase. The inability to actively degrade the dense matrix surrounding the cells prevented the chondrocytes from migrating, resulting in the formation of large aggregates of spherical-shaped cells through proliferation over the 14 days of study. Similarly, in a study by Tsai *et al* (41), chondrocytes embedded in collagen gel migrated <0.6 mm in distance after 14 days in the presence of bFGF. As the cell culture chamber of the microfluidic platform used in the present study was miniaturized (2 cm), the limited migration of the chondrocytes had a negligible impact on the results.

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