Basic fibroblast growth factor with human serum supplementation: enhancement of human chondrocyte proliferation and promotion of cartilage regeneration

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ABSTRACT

Introduction: The objectives of this study were to determine the optimum concentration of basic fibroblast growth factor (bFGF) in foetal bovine serum (FBS) or human serum (HS) supplemented medium for adult human nasal septum chondrocyte culture and to evaluate the potential of cartilage regeneration.

Methods: Dose effects of bFGF were evaluated from a range of 0.0 ng/ml to 10.0 ng/ml in the culture medium either supplemented with ten percent HS or ten percent FBS. Chondrocyte growth rate, viability and gene expression were evaluated. Cultured chondrocytes were then suspended in hydrogel for cartilage regeneration. Engineered cartilages were evaluated with standard histological staining and gene expression analysis.

Results: Our results showed that the chondrocyte growth rate increased in a dose dependent manner of bFGF until 5.0 ng/ml. This increment is further enhanced with ten percent HS supplementation. All cultured chondrocytes exhibited the same gene expression profile regardless of bFGF concentration and type of serum used. The histological staining and gene expression analysis of engineered cartilage after implantation showed characteristics similar to native cartilage.

Conclusion: bFGF with ten percent HS was able to accelerate the chondrocyte growth rate, provided more chondrocytes for therapeutic purposes and therefore minimised the amount of nasal septum cartilage needed to be harvested from patients. The combination of 5.0 ng/ml of bFGF and ten percent HS in the culture medium was safer and had less risk compared to FBS. It also demonstrated valuable implications on constructing high quality autologous cartilage for treating cartilage defects, especially in head and neck reconstructive surgery.

Keywords: basic fibroblast growth factor, cartilage regeneration, human chondrocytes, human serum, nasal septum cartilage

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INTRODUCTION

In the field of head and neck reconstructive surgery, nasal septum cartilage is commonly used as a source of autologous graft because of its structural integrity, easy accessibility by the surgeons, and low morbidity during harvest. The scarcity of available cartilage in the nose has resulted in a focus on a tissue engineering approach to generate a clinical size graft for replacing the damaged cartilage.[1,2] This technology requires an efficient cell culture system to multiply the limited number of autologous chondrocytes isolated from a biopsy, in a short period of time before any new cartilage can be generated.

Current cell culture techniques for human chondrocyte expansion are based on culture medium supplemented with 10% animal sera, such as foetal bovine serum (FBS). However, the use of FBS to generate new cartilage for human implantation should be seriously considered since FBS is prone to viral transmission and possible immune reaction against animal proteins present in the serum. It has been shown that there is an immune response against bovine serum proteins in burn patients receiving keratinocyte grafts cultured using FBS.[3] In order to overcome problems encountered on using animal sera, human serum (HS) should be investigated for its

potential of supporting the growth and differentiation of human chondrocytes. Previous studies with other types of human cells, such as cancellous bone-derived cells,[4] osteoblasts,[5] and bone marrow cells,[6] have demonstrated that HS promoted higher cell proliferation compared to FBS. In another study, human articular chondrocytes were shown to proliferate better when cultured with HS compared with other mammalian sera.[7] However, the full potential of HS on human nasal septum chondrocyte culture-expansion for hyaline cartilage tissue engineering has not yet been investigated.

Basic fibroblast growth factor (bFGF) has been recognised as an important factor in modulating chondrocyte mitogenesis and synthesis of cartilage matrix. bFGF promotes articular cartilage repair in humans by administration of exogenous source of bFGF into cartilage defects.[8] bFGF injected into the cartilage defect can induce chondrocyte proliferation and increase the formation of metachromatic-stained matrix at the defective area.[9] In monolayer culture, bFGF has been demonstrated to increase the proliferation of human septal[10,11] and auricular[12,13] chondrocytes. A high concentration of bFGF (100 ng/ml) has been used in serum-free medium culture experiments as a growth promoting agent for human chondrocytes,[10,13] whereas 10 ng/ml concentration of bFGF was used in 10% serum supplemented medium for human auricular chondrocytes study.[12] All these studies evaluated the effects of bFGF based on one concentration in the medium without knowing the optimal concentration of bFGF. It is important to determine the optimal concentration of bFGF in a dose-response study in order to avoid unnecessary additional costs onto the chondrocyte culture. Since the dose response of bFGF in 10% HS-supplemented medium is still unknown, the objectives of this study were to determine the optimum concentration of human recombinant bFGF in FBS or HS supplemented-medium for adult human nasal septum chondrocyte monolayer culture. In order to evaluate the potential of bFGF and HS supplementation on cartilage regeneration, we also investigated the use of culture expanded chondrocytes for cartilage reconstruction with hydrogel pluronic F-127. This hydrogel has been recognised as a better biomaterial compared to other scaffold materials by minimising the inflammatory response in the immunocompetent animal model.[14]

METHODS

Human nasal septum cartilage was obtained from six consenting adult patients, whose ages ranged from 25 to 55 years, after elective septoplasty. Patients are otherwise healthy, without any systemic disease such as diabetes mellitus or hypertension. These specimens would normally be discarded, and their use in this study has been approved by the Ethical Committee of Faculty of Medicine, National University of Malaysia. Each cartilage was cleaned from perichondrium, minced into small pieces and digested with 0.6% collagenase II (Gibco, Grand Island, NY, USA) at 37° C for 12 hours. After digestion, isolated chondrocytes were quantified with a haemocytometer (Weber Scientific International, Middlesex, England) and cell viability was determined with trypan blue vital dye (Gibco, Grand Island, NY, USA). Isolated chondrocytes were then seeded in six-well tissue culture plates (Falcon, Franklin Lakes, NJ, USA) as the primary culture (P0) with a density of 5,000 cells/cm^2 in an equal volume mix of Ham's F12 medium and Dulbecco's Modified Eagle Medium (F12: DMEM)(Gibco, Grand Island, NY, USA).

Dose response of human recombinant bFGF (Gibco, Grand Island, NY, USA) was evaluated in six different concentrations of 0.0 ng/ml, 0.2 ng/ml, 1.0 ng/ml, 3.0 ng/ml, 5.0 ng/ml and 10.0 ng/ml in the culture medium. Six nasal septum specimens were used for two series of experiments; one part with 10% pooled HS supplementation and the other with 10% FBS (Gibco, Grand Island, NY, USA; Catalogue number: 10270-098 and Lot: 40A1150K). In each serum group, all six different concentrations of bFGF were evaluated. HS was collected from the whole blood withdrawn from healthy donors. There were three women (aged 23 years, 28 years and 35 years, respectively) and three men (aged 24 years, 29 years and 34 years, respectively) who donated the blood. Whole blood (50 ml) was withdrawn from each individual and collected into serum tubes (Vaccute, Greiner Bio-one, Austria). About 25 ml serum was collected from each individual after the blood clotted. Serum from all donors was then pooled together and stored at -20°C before use. Therefore, the uniformity of the HS was maintained throughout the experiment. The serum donors and cartilage donors were not from the same person and the serum was not autologous serum. All cultures were maintained in 5% CO_2 incubator (Jouan, Duguay Trouin, SH) at 37°C with culture medium changed twice a week. Cell morphological features were examined every day by inverted light microscope (Olympus, Shinjuku-ku, Tokyo, Japan). When the P0 reached confluence, it was trypsinised with 0.05% trypsin-ethylenediaminetetraacetic acid (Gibco, Grand Island, NY, USA) and passaged three times (P1, P2 and P3). Chondrocyte growth rate (average increase of chondrocyte number in one day/cm^2) and cell viability in every culture stage were calculated for statistical analysis. Student's t-test was used to compare data between groups. Differences at the 5% level were considered significant.

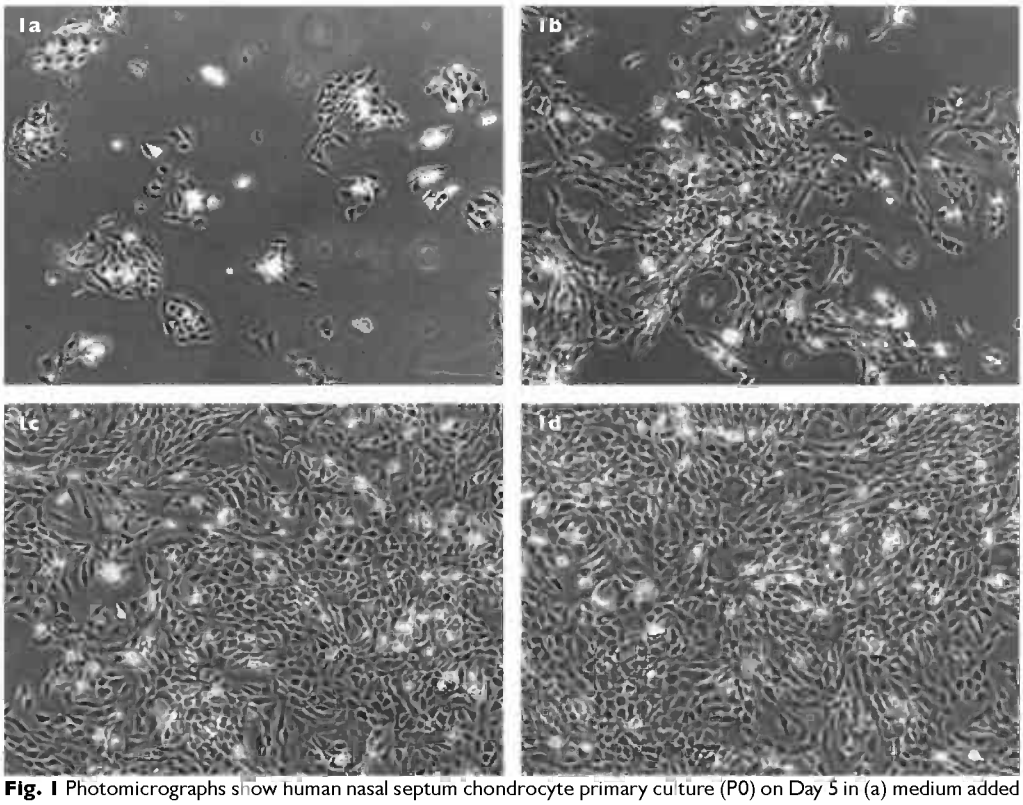

Fig. 1 Photomicrographs show human nasal septum chondrocyte primary culture (P0) on Day 5 in (a) medium added with 10% FBS; (b) medium + 10% HS; (c) medium + 10% FBS + 5.0 ng/ml bFGF; and (d) medium + 10% HS + 5.0 ng/ml bFGF; exhibited similar morphological features but different in cell density. (Magnification ×40).

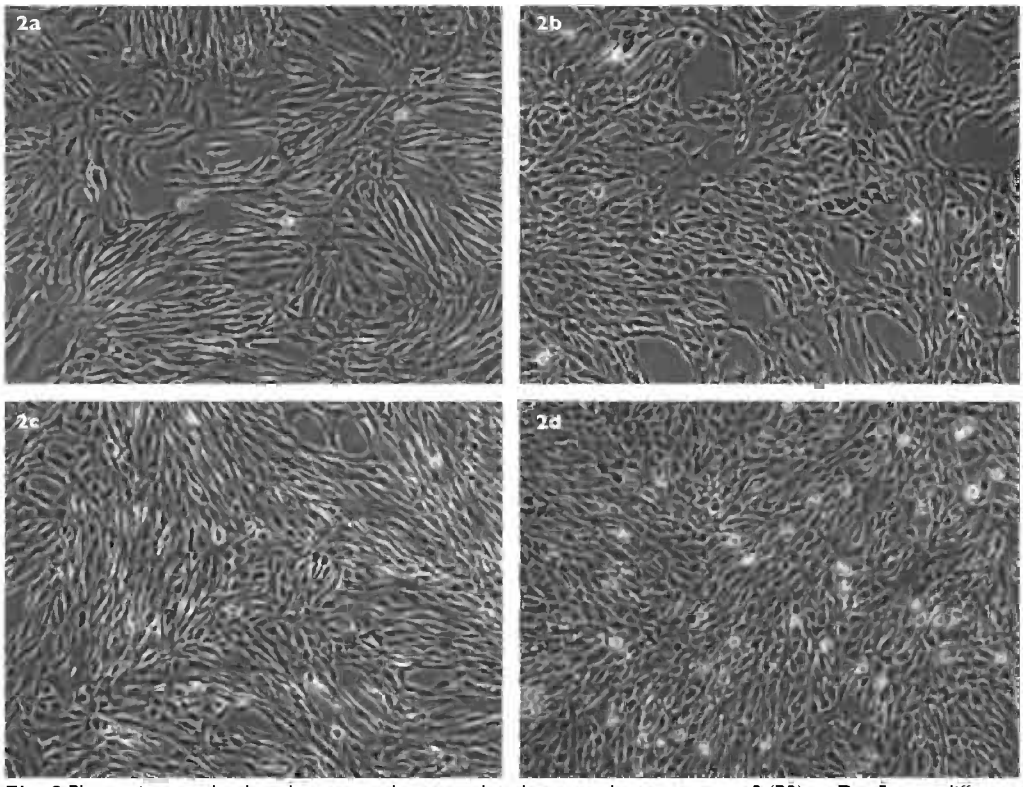

Fig. 2 Photomicrographs show human nasal septum chondrocyte culture at passage 3 (P3) on Day 5 were different in cell density in (a) medium added with 10% FBS; (b) medium + 10% HS; (c) medium + 10% FBS + 5.0 ng/ml bFGF; and (d) medium + 10% HS + 5.0 ng/ml bFGF. Chondrocytes cultured in medium containing 10% FBS (a & b) exhibited more elongated morphology compared to chondrocytes cultured in medium containing 10% HS (c & d). (Magnification ×40).

Chondrocyte phenotype gene expression analysis for type I and type II collagen was performed using one-step reverse transcriptase-polymerase chain reaction method (RT-PCR). Total RNA from cultured chondrocytes at various culture stages were extracted using TRI Reagent (Molecular Research Center, Cincinnati, OH, USA) according to the manufacturer's instructions. Yield and purity of the extracted RNA was determined by spectrophotometer (Bio-Rad, Hercules, CA, USA). The one-step RT-PCR reaction mix was prepared according to the manufacturer's instruction (Invitrogen, Carlsbad, CA, USA). Each reaction consisted of 100 ng total RNA and 10 pmol of each sense and antisense primers. Expression of human β-actin gene was used as control. The specific sense and antisense primers used were: type I collagen, 5′-AAGGCTTCCAAGGTCCCCCTGGTG-3′ and 5′-CAGCACCAGTAGCACCATCATTTC-3′, type II collagen, 5′-CTGGCAAAGATGGTGAGACAGGTG-3′ and 5′-GACCATCAGTGCCAGGAGTGC-3′; β-actin, 5′-CCGGCTTCGCGGGCGACG-3′ and 5′-TCCCGGCCAGCCAGGTCC-3′. The one-step RT-PCR was performed in a 9700 thermal cycler (Perkin Elmer, Norwalk, CT, USA) with a reaction profile of: cDNA synthesis for 30 minutes at 50°C; pre-denaturation for 2 minutes at 94°C; PCR amplification for 38 cycles with 30 s at 94°C, 30 s at 60°C and 30 s at 72°C. Subsequently, the PCR products were separated by electrophoresis on a 1.5% agarose gel visualised by UV transillumination (Vilber Lourmat, Marne La Vallee, France).

Four types of media were chosen for evaluating the potential of cartilage regeneration using the cultured chondrocytes, namely: medium + 10% FBS, medium + 10% HS, medium + 10% FBS + 5.0 ng/ml bFGF and medium + 10% HS + 5.0 ng/ml bFGF. Chondrocytes were plated in 175 cm^2 culture flasks (Falcon) at 5,000 cells/cm^2. Confluence cultures were trypsinised and the chondrocytes were harvested for engineered cartilage formation. Cultured chondrocytes from passage 3 in 175 cm^2 flasks were suspended into a 30% (weight/volume) co-polymer of polyethylene oxide and polypropylene oxide, Pluronic F-127 (BASF, Mount Olive, NJ, USA) at 4°C with a cell density of 3×10^7 cells/ml. The resulted admixer was then injected subcutaneously at the dorsal part of the nude mice at eight weeks of age under general anaesthesia (ketamine, xylazil and zoletil). Care of the nude mice was carried out following the animal facility guideline of the Animal Unit, Institute of Medical Research Malaysia. The engineered cartilages were harvested after eight weeks of in vivo implantation. The engineered cartilage was divided into two halves. One half was fixed in 10% phosphate buffered formalin (Fisher Scientific, Fair Lawn, NJ, USA) and processed into a paraffin-embedded block. Paraffin blocks were then sectioned and the slides sections were stained with Haematoxylin and eosin, and Safranin O staining. The other half of the excised tissue was digested with collagenase enzyme and total RNA was extracted for the one-step RT-PCR analysis, as mentioned earlier.

RESULTS

The average wet weight of the human nasal septum cartilage was 277 ± 74 mg. The average isolated viable chondrocytes was $2.4 \times 10^3 \pm 0.3 \times 10^3$ cells per mg of cartilage. Viability of the isolated chondrocytes was 96.1 ± 4.9%. Freshly-isolated human nasal septum chondrocytes adhered to six-well tissue culture plate on the second day of P0 and started to proliferate. Chondrocyte mitosis activity was accelerated with supplementation of bFGF and 10% HS. On Day 5 of primary culture, differences in chondrocyte density under the dose effect of bFGF can be clearly identified from the inverted microscope view. Medium added with 10% FBS without bFGF supported the lowest chondrocyte proliferation with small islands of chondrocyte growth on Day 5 of P0 (Fig. 1a). 10% HS without bFGF promoted higher chondrocyte proliferation with larger islands of chondrocytes growth and the culture reached about 50% confluence on Day 5 of P0 (Fig. 1b). Increase in chondrocyte mitosis activity was noted with higher concentrations of bFGF. On Day 5 of P0, chondrocytes cultured in medium supplemented with 5.0 ng/ml bFGF was almost confluence in both 10% FBS (Fig. 1c) and 10% HS (Fig. 1d) media. The chondrocyte morphological feature was neither affected by bFGF nor by the type of serum used. All chondrocytes were polygonal in shape and uniform in size at P0 (Fig. 1). The prominent mitogenic effects of bFGF and 10% HS were continued throughout the subsequent passages. At passage 3 (P3), chondrocytes cultured in media added with 10% HS maintained the polygonal feature (Figs. 2b & 2d), compared to chondrocytes cultured in media added with 10% FBS which exhibited fibroblastic elongation shape (Figs. 2a & 2c). Chondrocytes cultured in media supplemented with 5.0 ng/ml bFGF demonstrated higher mitosis activity and reached confluence earlier (Figs. 2c & 2d).

Human nasal septum chondrocytes growth rate (average increase in cell number per day/cm^2) increased with the dosage of bFGF from P0 to P3 (Fig. 3). bFGF showed a strong mitogenic effect starting from the lowest bFGF concentration (0.2 ng/ml) at P0 through all three passages (Fig. 3). The chondrocyte growth rate increased corresponding to the increase of bFGF concentration from 0.0 ng/ml to 5.0 ng/ml in the culture medium. Chondrocyte growth rate in the medium

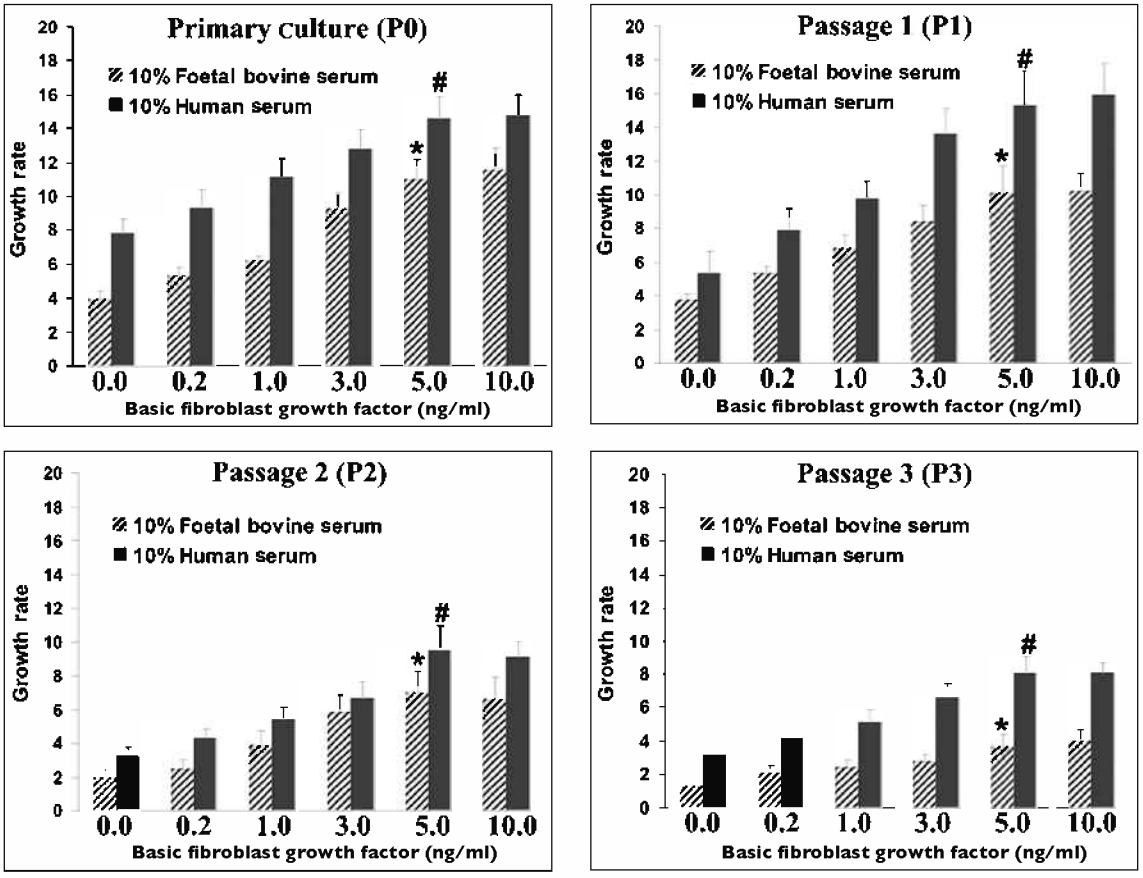

Fig. 3 Growth rate (average increase in cell number per day/cm^2) of human nasal septum chondrocytes cultured in medium supplemented with different concentration of bFGF either added with 10% FBS or 10% HS in primary culture (P0), passage 1 (P1), passage 2 (P2) and passage 3 (P3). Values were showed as mean ± SEM (n = 6). Growth rate in medium supplemented with 5.0 ng/ml bFGF was significantly higher than 0.0 ng/ml, 0.2 ng/ml, 1.0 ng/ml and 3.0 ng/ml bFGF in 10% FBS group (★) and 10% HS group (#).

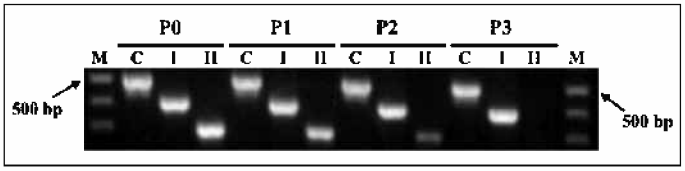

Fig. 4 One-step RT-PCR analysis on the phenotype gene expression of human nasal septum chondrocytes at primary culture (P0), passage 1 (P1), passage 2 (P2) and passage 3 (P3). Cultured chondrocytes demonstrated the same gene expression profile regardless of bFGF concentration and type of serum added into the medium. Lane M: 100 bp DNA marker. Lane C: β-actin gene fragment (Control; 495 bp). Lane I: Type I collagen gene fragment (396 bp). Lane II: Type II collagen gene fragment (294 bp).

supplemented with 5.0 ng/ml bFGF was significantly higher than medium supplemented with 3.0 ng/ml and the other three lower bFGF concentrations (Fig. 3). This chondrocyte growth kinetic pattern applied to both 10% HS and 10% FBS experiment groups (Fig. 3). No significant difference was noted for chondrocyte growth rate in medium supplemented with 10.0 ng/ml bFGF compared to 5.0 ng/ml bFGF. Chondrocyte growth rate decreased when the culture was passaged. The reduction of chondrocyte growth rate was more prominent at P2 and P3 (Fig. 3). Throughout the culture period, 10% HS supplementation demonstrated a higher chondrocyte growth rate compared to 10% FBS group with the same bFGF concentration (Fig. 3). All cultured chondrocytes scored a viability greater than 95% with no significant difference between the groups.

The chondrocyte expansion capacity (number of cell increased from initial seeding to P3) in the media was calculated by scoring the accumulative cell doubling from P0 to P3. Chondrocyte culture in medium supplemented with 5.0 ng/ml bFGF + 10% HS scored 17.2 ± 1.4 accumulative cell doubling (150,562–fold increase in cell number) from P0 to P3. Chondrocyte culture in medium supplemented with 5.0 ng/ml bFGF + 10% FBS scored 15.2 ± 1.7 accumulative cell doubling (37,641-fold increase in cell number) from P0 to P3. Chondrocyte culture in medium supplemented with 10% HS scored 11.6 ± 1.1 accumulative cell doubling (3,104-fold increase in cell number) from P0 to P3. Chondrocyte culture in medium supplemented with 10% FBS scored 10.3 ±

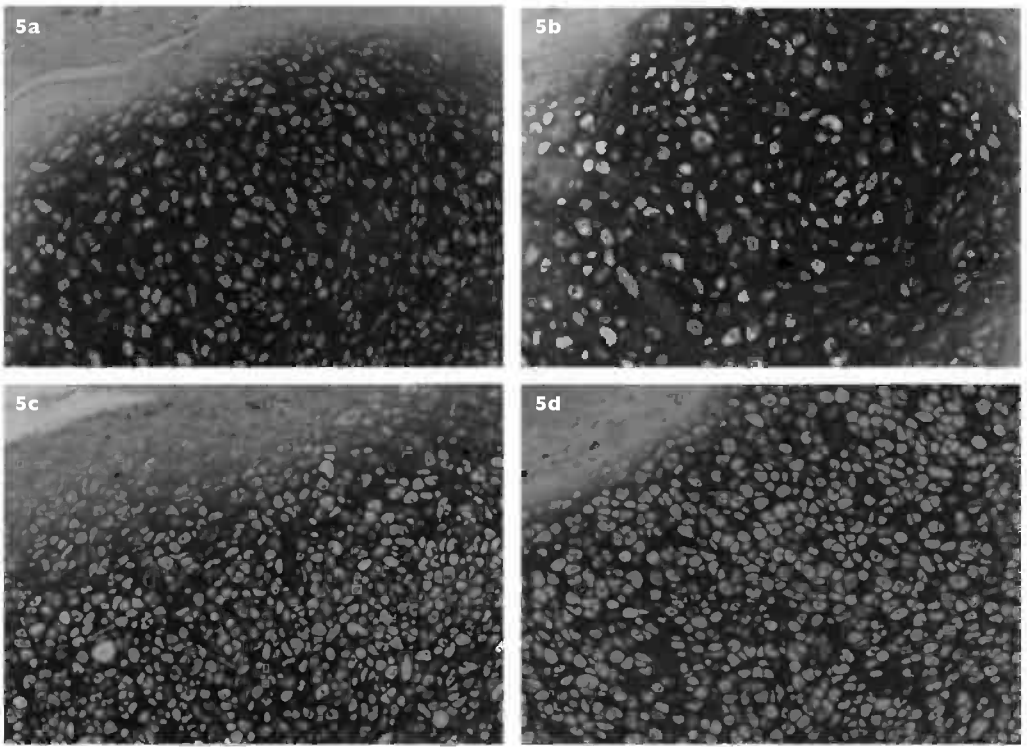

Fig. 5 Photomicrographs show engineered cartilage resulted from (a) medium added with 10% FBS; (b) medium + 10% HS; (c) medium + 10% FBS + 5.0 ng/ml bFGF; and (d) medium + 10% HS + 5.0 ng/ml bFGF (Safranin 0, ×40).

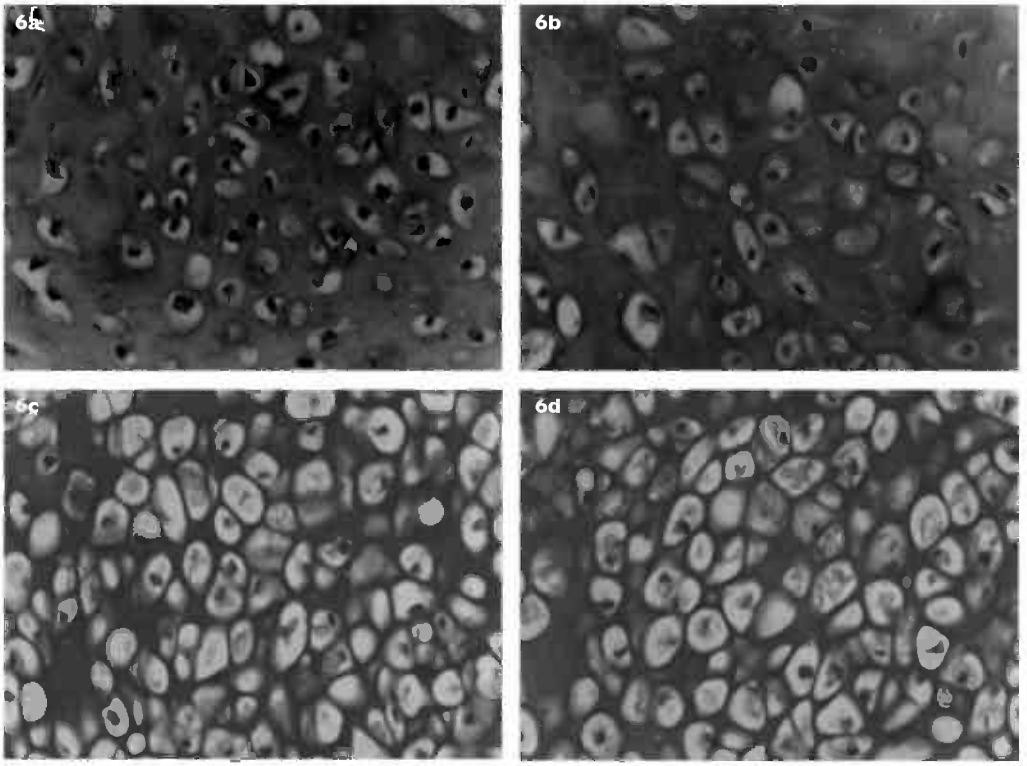

Fig. 6 Photomicrographs show engineered cartilage resulted from (a) medium added with 10% FBS; (b) medium + 10% HS; (c) medium + 10% FBS + 5.0 ng/ml bFGF; and (d) medium + 10% HS + 5.0 ng/ml bFGF (Haematoxylin & eosin, ×200).

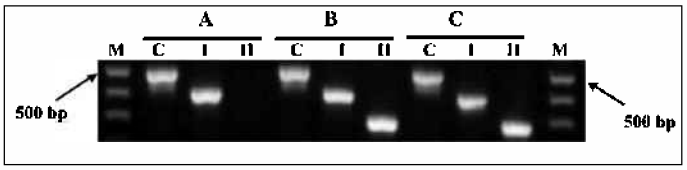

Fig. 7 One-step RT-PCR analysis on the phenotype gene expression of cultured chondrocytes from 175cm^2 culture flasks (A); engineered cartilage resulted from medium + 10% FBS and medium + 10% FBS + 5.0 ng/ml bFGF (B); and engineered cartilage resulted from medium + 10% HS and medium + 10% HS + 5.0 ng/ml bFGF (C). Lane M: 100 bp DNA marker. Lane C: β-actin gene fragment (Control; 495 bp). Lane I: Type I collagen gene fragment (396 bp). Lane II: Type II collagen gene fragment (294 bp).

1.2 accumulative cell doubling (1,261-fold increase in cell number) from P0 to P3. Chondrocytes cultured in medium supplemented with various concentration of bFGF, either supplemented with 10% HS or 10% FBS exhibited the same gene expression profile throughout the study (Fig. 4). At P0, cultured chondrocytes expressed both type II and type I collagen genes. In the subsequent passages, type II collagen gene expressed moderately at P1, expressed at low level at P2 and not detected at P3 (Fig. 4). Expression on type I collagen gene was maintained at the same level throughout all passages.

Engineered cartilage removed from nude mice after eight weeks of in vivo implantation were glistening white in colour and firm in consistency regardless of bFGF or type of serum supplemented. Safranin O staining (Fig. 5) was strongly positive in all tissue sections, correlating with abundant proteoglycans production in the extracellular matrix. A thin perimeter resembling perichondrium was also demonstrated on the histological sections. Engineered cartilage resulted from medium added with 5.0 ng/ml bFGF exhibited a higher cell density (Figs. 5c & 5d) compared to tissues resulted from only serum supplementation (Figs. 5a & 5b). Haematoxylin and eosin staining (Fig. 6) generally demonstrated engineered cartilage consisting of evenly-spaced lacunae cells embedded in the basophilic matrix. The lacunae cells were varied from round to oval with slight polymorphism. At higher magnifications, higher cell density was clearly noted in engineered cartilage resulted from medium added with 5.0 ng/ml bFGF (Figs. 6c & 6d), compared to tissues resulted from only serum supplementation (Figs. 6a & 6b).

Cultured chondrocytes (P3) harvested from 175 cm^2 flasks for cartilage regeneration exhibited negative expression on type II collagen gene, regardless of type of serum used and bFGF supplementation (Fig. 7). After eight weeks of in vivo implantation, cells isolated from each engineered cartilage were analysed for chondrocyte phenotype gene expression. Results showed that all engineered cartilages (regardless of type of serum used and bFGF supplementation) re-expressed type II collagen gene after losing it in monolayer culture (Fig. 7). Type I collagen gene was also expressed in all the engineered cartilages (Fig. 7). No differences on the gene expression pattern was noted for all four tested groups (medium + 10% FBS, medium + 10% HS, medium + 10% FBS + 5.0 ng/ml bFGF, and medium + 10% HS + 5.0 ng/ml bFGF).

DISCUSSION

Our results demonstrated that healthy and viable chondrocytes can be isolated from the adult human nasal septum cartilage and the chondrocytes can proliferate well in monolayer culture in all the culture groups. This result concurred with Rotter et al's study and thus further supports the idea of using human nasal septum chondrocytes as a starting material for cartilage tissue engineering.[15] The cultured chondrocytes in our study generally demonstrated a flattened morphology with cell elongation in a long-term monolayer culture. The fibroblastic character was even more prominent if bFGF was added as a growth promoting agent in the culture medium, as also shown in animal and young human auricular chondrocytes cultures.[12,16] These fibroblastic features had been described by Homicz et al as dedifferentiation, and correlated with reduction of type II collagen production and glycoaminoglycan synthesis.[17]

Gene expression analysis by one-step RT-PCR in the results correlated well with the chondrocyte dedifferentiation process in multiple passages by the gradual reduction of type II collagen gene expression and high expression on type I collagen gene (Fig. 4). Modification on the cell cytoskeleton in the monolayer culture was suggested as the reason for chondrocyte dedifferentiation.[18] By returning the cultured chondrocytes into a three-dimensional culture such as alginate system, the differentiation phenotypes of chondrocytes can be promoted.[19] Although the utilisation of HS in our study managed to enable a more polygonal shape of cultured chondrocytes until P3, even with the inferences of bFGF (Fig. 2), HS did not promote a higher expression of type II collagen gene in cultured chondrocytes as demonstrated by one-step RT-PCR analysis (Fig. 4).

Chondrocyte growth rate was strongly influenced by the bFGF concentration. A significant difference in chondrocyte growth rate was noted from P0 to P3 even in the concentration as low as 0.2 ng/ml bFGF in the medium. The proliferative effects of bFGF reached plateau at 5.0 ng/ml, and no or little incremental growth rate was noted at higher

concentration. Therefore, our results in this study concluded that 5.0 ng/ml of bFGF is the optimum dose and a further increase in bFGF concentration will only add unnecessary costs. HS promoted a higher increase in growth rate of cultured chondrocytes from all tested bFGF concentration, compared with FBS. Tallheden et al showed that HS contained higher levels of epidermal-derived growth factor and platelet-derived growth factor-AB, compared to FBS, and promoted higher proliferation of human articular chondrocytes.[20] In addition, HS may have caused a greater up-regulation of growth factor receptors in chondrocytes, with more specific binding of growth factors in homologous serum to chondrocyte receptors, and thus enhanced receptor functions involved in cellular mitosis activities.

The capacity of chondrocyte expansion was the highest when the medium was supplemented with 5.0 ng/ml of bFGF + 10% HS. Thus, this result is very meaningful when the technology is transformed for clinical use, as a surgeon can easily harvest 100 mg of nasal septum cartilage from patients without causing any deformity of the nose architecture. As many as 2.4×10^5 viable chondrocytes can be isolated, and a 150,562-fold increase in chondrocyte number is expected when cultured until P3. As shown in our results, 3.6×10^{10}, or 36 billion chondrocytes ($2.4 \times 10^5 \times 150{,}562$), can be obtained at P3 for cartilage engineering purposes. This number of chondrocytes is more than enough to treat any cartilage defect. With these results, HS added together with 5.0 ng/ml of bFGF has demonstrated to have a superior potential compared to animal serum, for human chondrocyte culture expansion for cartilage tissue engineering. Moreover, the use of animal sera for human chondrocyte culture and cartilage engineering intended for human use introduces the risk of viral transmission and immunogenical response. Substitution of animal serum with HS will avoid these problems and bring human cartilage tissue engineering nearer to clinical application.

Cultured chondrocytes mixed well with pluronic F127 in a liquid state at a cold temperature. Later, when warmed up to physiological temperatures (dorsum part of the nude mice), pluronic F127 transformed into a thick semisolid gel and provided a three-dimensional environment that allowed chondrocytes to re-express type II collagen gene (Fig. 7) and promote matrix production (Fig. 5) in in vivo implantation. Thus, pluronic F127 has provided a suitable in vivo environment for dedifferentiated chondrocytes to re-differentiate by expressing type II collagen gene and produce cartilage matrix in the explants tissues. However, pluronic F127 does not provide any permanent shape, and the engineered cartilage appeared as a lump structure under the skin of nude mice after the injection. Our data also showed cells isolated from the engineered cartilages expressed type I collagen gene. This may be due to the progenitor cells in the engineered cartilage that are still in the process of developing into mature chondrocytes.[21] Histological analysis on all the harvested engineered cartilages demonstrated abundant glycoaminoglycan production from the lacunae cells and exhibited comparable quality with native human nasal septum cartilage regardless of bFGF and the type of serum used. These results were in agreement with other reports showing that pluronic F127 promoted reconstruction of high quality cartilage with swine and human auricular chondrocytes.[14,22]

In conclusion, basic FGF with the supplementation of 10% HS was able to accelerate chondrocyte growth rate of more than 10% FBS. Medium supplemented with 5.0 ng/ml of bFGF and 10% HS provided more chondrocytes for therapeutic purposes and therefore minimised the amount of nasal septum cartilage needed to be harvested from patients. The combination of 5.0 ng/ml of bFGF with 10% HS in the culture medium was safer and less risky, compared to FBS. Medium added with 5.0 ng/ml of bFGF and 10% HS has also demonstrated valuable implications on constructing high-quality autologous cartilage for treating cartilage defects, especially in head and neck reconstructive surgery.

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