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Transforming Growth Factor Beta 3 Induced Human Adipose-Derived Stem Cells for Auricular Chondrogenesis

(Mengubah Faktor Pertumbuhan Beta 3 Aruhan Stem Asal Manusia Terbitan Adipos untuk Aurikul Kondrogenesis)

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ABSTRACT

The limitation of self-repair and proliferation capacity of chondrocytes in cartilage reconstruction lead to alternative search of cell source that can improve the auricular regeneration. Human adipose-derived stem cells (HADSC) are an alternative cell source that have unique characteristics to self-renew and differentiate into various tissues making it suitable for cell therapy and tissue engineering. This study aimed to examine the chondrogenic differentiation potential of (HADSC) in monolayer culture by the presence of different transforming growth factor beta's, TFG-β1, -β2 and -β3. HADSC at passage 3 (1.5 × 10^5 cell/mL) were cultured in chondrogenic medium containing 5 ng/mL of different transforming growth factor beta's, TFG-β1, -β2 and -β3 for 7, 14 and 21 days. Data analysis was evaluated based on the growth rate of cells, cells morphological changed, production of collagen type II and glycosaminoglycan sulphate (sGAG). The quantitative RT-PCR was carried out to determine the chondrogenic, fibrogenic and hypertrophic gene expression levels. Differentiation of HADSC into chondrocytes using TFG-β indicates the occurrence of the chondrogenesis process. The best chondrogenic differentiation was observed in HADSC induced by TFG-β3 through the chondrocytes-like cells morphology with cells aggregation and high production of proteoglycan matrices compared to other TGF-βs groups. Additionally, the expression of chondrocytes-specific genes such as Type II collagen, Aggrecan core protein, Elastin and Sox 9 was high. In conclusion, this study has showed that TGF-β3 is the potential growth factor in producing chondrogenic cells for auricular cartilage tissue engineering.

Keywords: Chondrocytes; chondrogenic differentiation; human adipose-derived stem; induction; tgf-β3

ABSTRAK

Kekurangan kapasiti pembaikan dan proliferasi kondrosit dalam pembentukan semula tisu rawan menyebabkan penyelidikan kini cenderung untuk mencari sumber sel baru yang dapat membantu memperbaiki tisu rawan aurikul dengan lebih baik. Sel stem adipos manusia (HADSC) dilihat sebagai sumber sel yang mempunyai ciri-ciri unik, boleh membahagi dengan sendiri dan membeza kepada pelbagai tisu, menyebabkan ia sesuai digunakan dalam terapi sel dan kejuruteraan tisu. Kajian ini bertujuan menilai tahap pembezaan kondrogeniknya secara monolapisan kultur dengan kehadiran faktor pertumbuhan transformasi beta yang berbeza iaitu TFG-β1,-β2 dan -β3. HADSC pada pasaj 3 (1.5 10^5 sel/mL) dikultur di dalam media kondrogenik yang mengandungi 5 ng/mL faktor pertumbuhan transformasi beta yang berbeza iaitu TFG-β1,-β2 dan -β3 selama 7, 14 dan 21 hari. Analisis data dinilai berdasarkan kepada kadar pertumbuhan sel, perubahan morfologi sel, pengeluaran kolagen jenis II dan glikoaminoglikan fosfat (sGAG). Kuantitatif RT-PCR dijalankan untuk menentukan kadar ekspresi gen kondrogenik, fibrogenik dan hipertrofik. Pembezaan HADSC kepada kondrosit menggunakan TFG-β menunjukkan berlakunya proses kondrogenesis. Pembezaan kondrogenik yang terbaik dilihat pada HADSC yang diaruhkan oleh TFG-β3 melalui perubahan morfologi dengan pembentukan sel agregasi dan penghasilan matrik proteoglikan yang tinggi apabila dibandingkan dengan kumpulan TFG-β yang lain. Tambahan pula, pengekspresan gen spesifik kondrogenik iaitu Kolagen jenis II, Elastin, Protien teras agrekan dan Sox 9 menunjukkan peningkatan. Kesimpulannya, kajian ini telah menunjukkan TFG-β3 berpotensi sebagai faktor pertumbuhan yang berupaya membantu penghasilan sel yang bercirikan kondrosit aurikular bagi strategi kejuruteraan tisu rawan aurikular.

Kata kunci: Aruhan; kondrosit; pembezaan kondrogenik; sel stem adipos manusia; tgf-β3

INTRODUCTION

Congenital or acquired deformities of the external ear have significant negative impact on patients. This defect also involves the formation of abnormal or absent of duct connects to the middle ear (Luquetti et al. 2012). At present, the most commonly used treatment for deformities of the external ear is the ear-shaped prosthetics or hand-carved autologous costal cartilage graft (Cho et al. 2007). The harvesting of costal cartilage procedure causes the morbidity at the donor site and has a risk of infection

after the surgery (Ishak et al. 2011; Zhang et al. 2014). The use of prosthetic ear has complication on extrusion of the plastic synthetic graft (Ishak et al. 2015). Hence, the development of alternative ear construct is needed to treat the defect by improving the clinical outcome of external ear reconstruction (Ruszymah et al. 2007).

In order to solve these problems, tissue engineering has been introduced. In recent years, tissue engineering has given a good development in the treatment of external ear defect in which the formation of new cartilage is produced *in vitro* and *in vivo* (Hohman et al. 2014). However, there is a limitation to this process because of the large amount of chondrocytes needed to form the human size external ear cartilage in a given time. Previous studies have found that the characteristic of chondrocytes will gradually disappear when chondrocytes were cultured in 2D culture flasks *in vitro* for too long (Kamil et al. 2003). Thus, stem cells with high renewability and chondrogenic ability are targeted as a new alternative cell source for cartilage tissue engineering.

Human adipose-derived stem cells (HADSC) are mesenchymal stem cells that are potent in regenerative medicine and tissue engineering fields. HADSC are easily acquired and isolated from fatty tissue (Hui et al. 2012; Zhao et al. 2012). Additionally, HADSC have multi potency characteristics, especially in chondrogenic differentiation capabilities that are similar to bone marrow stem cells (De Ugarte et al. 2003). However, differentiation of HADSC into the chondrocytes is dependent on the appropriate biological and micro-environmental signals. Therefore, to produce cartilage from HADSC, factors such as media, matrix composition, chondrogenic differentiation factors, oxygen tension and mechanical environment need to be determined. Additionally, the factors that affect the process of the chondrogenesis in stem cells are still unclear.

Generally, members of transformation growth factors and additional growth factors or hormones such as Fetal Bovine Serum (FBS), Insulin-Transferrin- Selenium (ITS), ascorbic acid, Insulin-like Growth Factor-1 (IGF-1) and dexamethasone were used in chondrogenic induction media which could affects chondrogenic growth and differentiation in stem cells (Hamid et al. 2012). Previous studies have shown that additional growth factors such as FBS can stimulated cell growth because it contains many growth factors, cytokines and peptides that show significant diversity (Awad et al. 2003). The additional growth factor such as ITS is a serum replacement that reduces the variety of serum variation in growth and cell differences (Chua et al. 2005) and prevents the differentiation of monoculture (Liu et al. 2014). In addition, ascorbic acid plays a role in modulating the growth and diffusion of cells by stimulating monolayer culture cell proliferation and enhancing the type of collagen genes expression in a short period of time (Awad et al. 2003). The IGF-1 factor is believed to enhance the effect of TGF-β3 on chondrogenic induction and its involved in regulatory metabolism (Zhou et al. 2016). Meanwhile, dexamethasone also plays a role in the process of stem cell chondrogenic by promoting differentiation into chondrocytes. Meanwhile, previous studies have also shown that the members of transformation growth factors comprising various peptide isomers which play a role in controlling the development of the process of the chondrogenesis (Awad et al. 2003). However, the role of each members of transformation growth factor in modulating the process of chondrogenesis is still unclear.

This study focuses on one of the factors mentioned previously which is the inductive growth factor added in the chondrogenic medium to induce HADSC differentiation. The beta-growth factor (TGF-β) consisting of TGF-β1, TGF-β2 and TGF-β3 is one of the growth factors widely used in the mesenchymal stem cell chondrogenic process (Crecente-Campo et al. 2017). However, each TGF-β showed different effects on chondrogenesis on stem cells in terms of regulatory control, cell viability/proliferation, cell differentiation and extracellular matrix production. Hence, this study hypothesised that human adipose stem cells have differentiating capabilities to chondroblast with the presence of secondary family transformation growth factors in the chondrogenic media and each TGF-β has a special regulatory effect in chondrogenic process of stem cells.

Materials and Methods

Ethical Approval

The ethical approval to use HADSC in this study was obtained from the Research and Ethics Committee of Universiti kebangsaan Malaysia Medical Centre (Approval code of 02-01-02-SF0976). All samples used in this study were accompanied with informed consent from patients at Universiti Kebangsaan Malaysia.

Isolation of Human Adipose Derived Stem Cells (HADSC)

Briefly, human adipose sample from caesarean operation was minced into small pieces and washed with PBS containing 1% of antibiotic/antimycotic solution (Gibco, USA). The sample was digested using 0.3% collagenase type I (Worthinghon, USA) at 37°C for 30 min. After digestion, the pelleted cells were re-suspended in DMEM medium containing 10% fetal bovine serum (FBS; Invitrogen), 1% antibiotic-antimycotic (Gibco-Invitrogen), 1% glutamax (Gibco- Invitrogen), 1% vitamin C (Sigma-Aldrich, St Louis, MO) and incubated at 37°C with 5% CO_2. Once the cultured cells reached 80 to 90% confluency, cells were detached with 0.125% trypsin-EDTA and passaged. Cells at passage 3-4 were used for subsequent experiments.

Chondrogenic Differentiation of Human Adipose Derived Stem Cells

Cell culture at passage 3 which reached 80% confluency was trypsinized using trypsin EDTA 0.125% (TE). A total of 1.5×10^5 cells/mL was suspended in a chondrogenic induction medium containing either 5 ng/mL of TGF-Beta 1 or TGF-Beta 2 or TGF-Beta 3 and cultured into a six well plate. Cells were incubated in 5% CO_2 incubator at

37ºC for three weeks. Medium was changed every three days. The cultured cells were assessed for growth rate, immunocytochemistry staining, sGAG and qRT-PCR.

GROWTH RATE

Cell morphology changes were evaluated using light inverted microscope at the first, second and third weeks. Cells were cultured in six wells plate with a cell density of 150,000 cells per well over three weeks. The cell growth was measured at first, second and third week using Alamar Blue assay. A total of 100 µL Alamar Blue reagents was added to each well containing 1000 µL medium culture with a ratio of 1:10 and incubated for 4 h at 37ºC in 5% CO_2 incubator under dark condition. After 4 h of incubation, 100 uL of the treated medium was dispended into each well on the 96-wells plate. The value of the absorbance of each well was read at the wavelength of 570 nm.

IMMUNOCYTOCHEMISTRY STAINING

Cultured cells were rinsed using PBS × 1 three times before being fixed with 4% paraformaldehyde solution for 1 h at 4ºC. The cells were then rinsed with PBS × 1 cold-tempered twice. Subsequently, the cells were treated with Triton X-100 0.1% solution for 5 min and rinsed using Tween 20 0.1% solution. Cells were blocked using 10% goat goats for 1 h at 37ºC in dark condition. The primary antibody solution was added to the well and incubated overnight at 4ºC. After that, cells were washed using Tween 20 0.1% solution, PBS × 1 for 10 min each. The cells were then rinsed using 1% goat serum and secondary antibodies were added to the well. Cells were incubated for 2 h at 37ºC. After that, the cells were washed using a Tween 20 0.1% solution and PBS × 1 for 5 min each. The staining of the nucleus was continued by adding a solution for nucleus staining and incubated at room temperature for 20 min. Subsequently, the cells were washed using PBS × 1 and wrapped in aluminium foil to prevent exposure to light until observed under a light microscope.

sGAG AND DNA ANALYSES

The samples were digested using digestion buffer and proteinase K under occasional vortexing at 55°C. The digested samples were assayed for DNA content using PureLink Genomic® DNA kits (Invitrogen, UK) according to the manufacture's instruction. Measurements were made at 535 nm. Proteoglycan content was determined by quantifying the amount of sulphated glycosaminoglycans (sGAG) in samples using the Blyscan Kit (Biocolor, UK) according to the manufacturers' instructions. Measurements were made at 656 nm. The standard curve was generated with bovine trachea chondroitin sulfate A.

QUANTITATIVE GENE EXPRESSION BY REAL-TIME PCR

Total RNA of samples were extracted using TRI reagent (Molecular Research Centre, Cincinnati, OH) and subsequent synthesis of cDNA with an Invitrogen SuperScript III First-Strand cDNA kit (Invitrogen) according to the manufacturer's instructions. Real-time PCR reaction was performed with SYBR Green fluorescent dye (SYBR Select Master Mix, Applied Biosystems) according to the manufacturer's recommendation. The real-time PCR reactions were carried out with pre-denaturation at 95°C for 3 min, followed by 40 cycles of denaturation with 10 s at 95°C, annealing and extension for 30 s at 61°C using Bio-rad iCycler. The PCR cycles were followed by a melting curve analysis to determine the specificity of the PCR products. The expression level of each targeted gene was normalized to glyceralde- hyde-3-phosphate-dehydrogenase gene (GAPDH) and was then calculated for statistical analysis. The primer sequences for collagen type II, elastin, aggrecan core protein, and sox9 were listed in Table 1. Primers were chosen from GeneBank data base sequences corresponding to the specific gene Accession Number, according to previously described (Goh et al. 2017).

STATISTICAL ANALYSIS

Data were assessed as mean± SEM (standard error of the mean). Obtained results were analysed using Student's t-test using SPSS 20.0 (SPSS Inc., Chicago) and P values < 0.05 were considered significant.

RESULTS AND DISCUSSION

EFFECT OF TGF-BS ON GROWTH RATE AND MORPHOLOGY OF HADSC

Figure 1 shows the total cell number of HADSC induced with TGF-β1, -β2 and -β3. The total cell number was not significantly difference in TGF-β3 when day 21 compared to day 7 and day 14. In TGF-β1 induced group, the total cell number on day 14 and day 21 was significantly ($p<0.05$) increase compared to day 7. The HADSC induced with TGF-β2 showed significant ($p<0.05$) higher cells number on day 14 compared to day 7 and day 21. In contrast, the total cell number of HADSC control group (HAD) was significantly ($p<0.05$) increased on day 21. However, the total number of cells in human auricular chondrocyte control group (NAU) showed no significant difference on day 14 compared to day 7. The cell morphology in each groups were showed in Figure 2. HADSC control group (HAD) showed no morphological changes with fibroblastic feature throughout 3 weeks culture. Whereas, the human auricular chondrocyte control (NAU) showed polygonal cells feature and began to show chondrogenic cell aggregation from day 7. Chondrogenic cell aggregation was also demonstrated in groups induced with TGF-β1 and β3. During the first 7 day culture, HADSC induced with TGF-β1 and β3 from fibroblastic feature transformed into polygonal shape and cells started to aggregate together. Cell aggregation was increased and formed large cell clumps with longer culture period (day 14 and day 21).

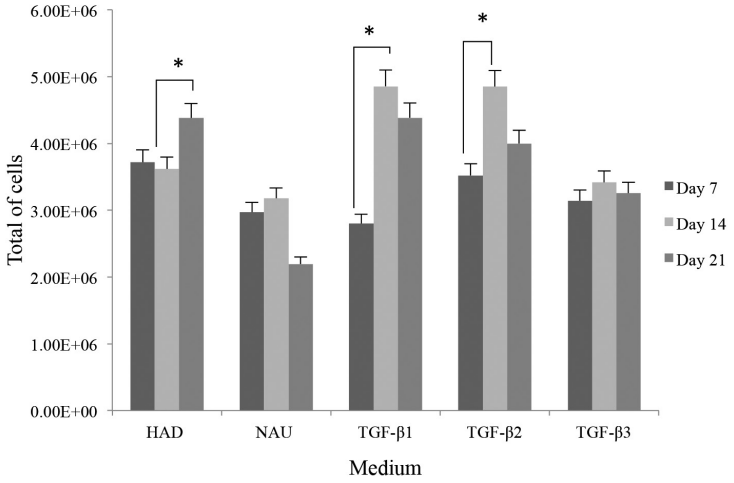

FIGURE 1. The total cell number of HADSC induced with TGF-β1, TGF-β2 and TGF-β3 on day 7, 14 and 21. HADSC (HAD) and human auricular chondrocyte (NAU) shown as control groups. Symbols * denote significant differences between culturing days in each group ($p<0.05$). The value showed mean ± SEM, $n=6$

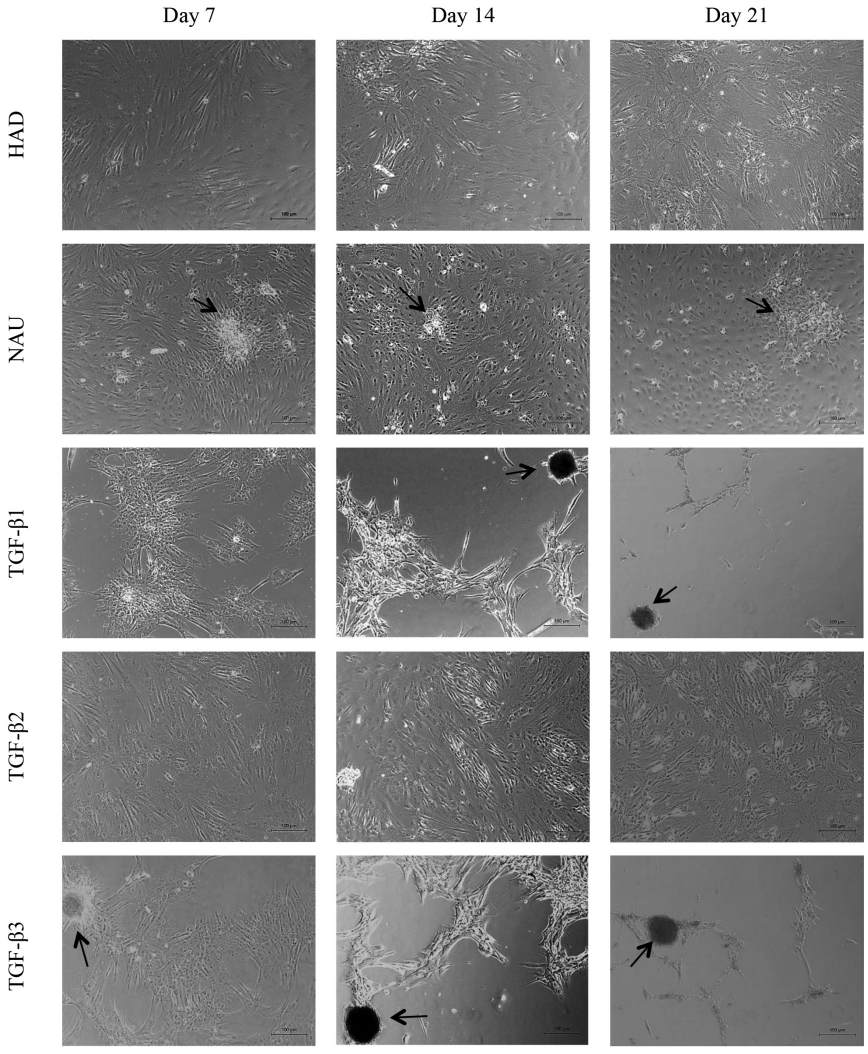

FIGURE 2. The morphological changes of HADSC induced with TGF-β1, TGF-β2 and TGF-β3 on day 7, 14 and 21. HADSC (HAD) and human auricular chondrocyte (NAU) shown as control groups. The (↑) sign indicates cell aggregation (100× magnification)

While, HADSC induced with TGF-β2 did not show cell aggregation, the cells remained fibroblastic feature till day 21 culture.

Our finding suggested that cell aggregation was the initial sign of chondrogenesis process. This happen only in HADSC induced with medium containing TGF-β3 or TGF-β1. In consistent with previous studies, the fibroblastic stem cell morphology was transformed to polygonal shape and started cell aggregation before the cartilage extracellular matrix was deposited in the tissue (Wilson et al. 2011). After cell aggregation, the adhesion molecules on the surface of cells decreases as the cells soon apart from each other by the production of extracellular matrix with continuation of the differentiation process (Puetzer et al. 2010). Our results were also reported in other studies that cell aggregation was observed in human mesenchymal stem cells at day 14 in the chondrogenic medium containing TGF-β1 and dexamethasone (Indrawattana et al. 2004). Study by Indrawattana et al. (2004) reported that chondogenesis in human mesenchymal stem cells was better and faster when induced in chondrogenic medium containing TGF-β3, IGF-1 and BMP (Puetzer et al. 2010).

EFFECT OF TGF-BS ON COLLAGEN TYPE II PRODUCTION IN HADSC

The production of collagen type II was detected by green-coloured fluorescent antibodies (Figure 3(A)) and blue DAPI staining was used to show the cell nucleus (Figure 3(B)). Human auricular chondrocyte control group (NAU) served as the positive control group for collagen type II staining. Based on this immunocytochemistry staining result, HADSC induced with TGF-β showed positive collagen type II production compared to HADSC control (HAD). The collagen type II staining was more intent in group induced with TGF-β3 compared to TGF-β1 and TGF-β2. The collagen type II staining was positive on monolayer cells as well as aggregation. The HADSC induced TGF-β1 showed reduce in cells aggregation and number at culture on day 21. HADSC induced with TGF-β2 just demonstrate mild collagen type II staining throughout 21 days culture. HADSC control group did not showed positive staining for collagen type II.

The production of collagen type II in HADSC induced with TGF-β3 showed more positive compared to HADSC induced with TGF-β1 and TGF-β2. This results were supported by previous studies that bone marrow stem cells and adipose-derived stem cells cultured in chondrogenic medium containing TGF-β3 and dexamethasone produced more collagen type II compared to medium containing TGF-β1 and TGF- β2 (Estes et al. 2006). HADSC induced with TGF-β1 shown more intent of collagen type II immunostaining on day 14 which was consistent with a report by Huang et al. (2016) claimed that the production of collagen type II in the hMSC cultured with TGF-β1 was higher on day 14 (Barry et al. 2001; Huang et al. 2016).

EFFECT OF TGF-BS ON THE PRODUCTION OF GLYCOSAMINOGLYCAN PHOSPHATE (SGAG) IN HADSC

In biochemical studies, cell density was determined by total DNA content and the amount of GAG phosphate production was measured by Blyscan's assay. The ratio of sGAG/DNA was then calculated to measure the amount of cartilaginous protein produce per amount of DNA. HADSC control group (HAD) showed no significant different of sGAG throughout 3 weeks culture. HADSC induced with TGF-β3 showed the highest sGAG/DNA compared to other groups for culture on day 14 and 21. These sGAG/DNA values for HADSC induced with TGF-β3 on day 14 and 21 were significantly (p<0.05) higher than other groups. This was contributed by lower DNA and consistent high synthesis of collagen type II throughout 3 weeks culture. However, other groups were showed significantly (p<0.05) reduced of sGAG/DNA value on day 14 and 21. Our findings was supported by previous studies that showed the production of GAG was lower in hMSC cultured in chondrogenic medium containing dexamethasone and TGF-β1 on day 7, 14 and 21 compared to chondrogenic medium containing TGF-β3 and TGF-β2 (Indrawattana et al. 2004). The higher sGAG/DNA production in HADSC induced with TGF-β3 suggested that TGF-β3 is the best growth factor in stem cell chondrogenic differentiation and production of cartilaginous extracellular matrix (ECM).

EFFECT OF TGF- BS ON THE EXPRESSION OF CHONDROGENIC GENE IN HADSC

Quantitative RT-PCR analysis was performed on HADSC induced with TGF-βs to assess the level of genes expression in HADSC control group (HAD), human auricular chondrocyte control group (NAU), HADSC induced with TGF-β1, TGF-β2 and TGF-β3 on day 7, 14 and 21 cultures. The expression of Type II collagen gene (Figure 5(A)) was showed significantly (p<0.05) higher expressed by 6.0 and 2.7 folds on day 14 compared to day 7 cultures in HADSC induced with TGF-β1 and TGF-β2, respectively. Meanwhile, Type II collagen gene expression in HADSC induced with TGF-β3 was high throughout 3 weeks culture and showed no significant difference in various time points. However, HADSC induced with TGF-β3 showed the highest Type II collagen gene expression compared to other groups. For the expression of Elastin (Figure 5(B)), gene expression was observed to be significantly (p<0.05) increased in HADSC induced with TGF-β1 and TGF-β3 on the day 21 culture compared to day 7 and day 14 culture. HADSC induced with TGF-β3 was found to exhibit the highest Elastin gene expression compared to other groups. Meanwhile, Elastin expression in HADSC induced with TGF-β2 was moderate throughout 3 weeks culture period. Gene expression of Aggrecan core protein was showed significantly (p<0.05) increased by 3.0, 11.0 and 7.0 fold in the HADSC induced with TGF-β1, TGF-β2 and TGF-β3 on day 14 compared to day 7 (Figure 5(C)). Additionally, HADSC induced with TGF-β3 showed significantly (p<0.05) increased in Aggrecan core protein gene expression on day

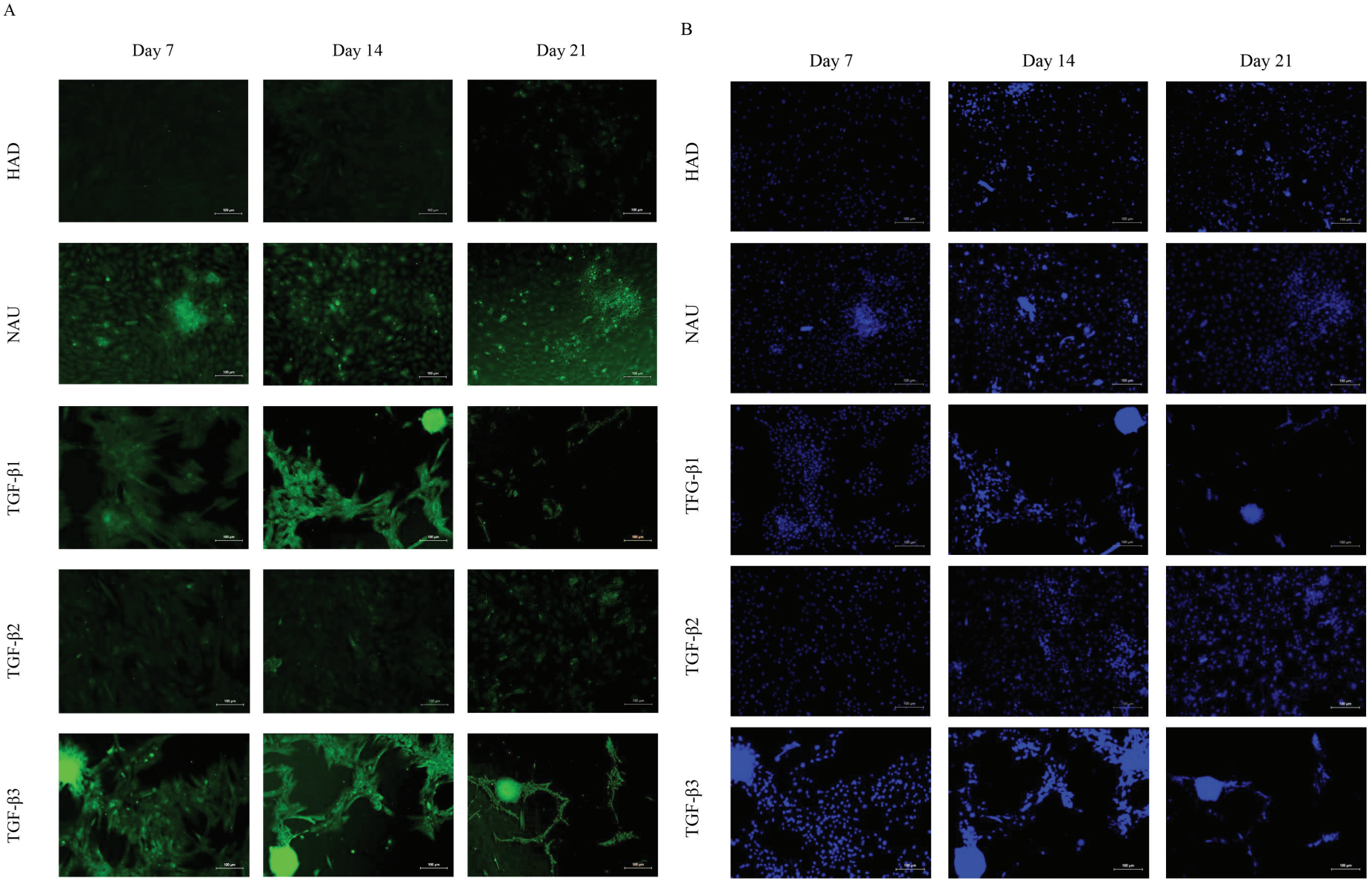

FIGURE 3. The production of collagen type II on day (i) 7; (ii) 14; and (iii) 21 culture. The production of collagen type II was stained with green colour. The cell nucleus was stained with DAPI blue for HADSC control (HAD), Human auricular chondrocyte control (NAU), HADSC induced with TGF-β1, HADSC induced TGF-β2 and HADSC induced TGF-β3. (100× magnification)

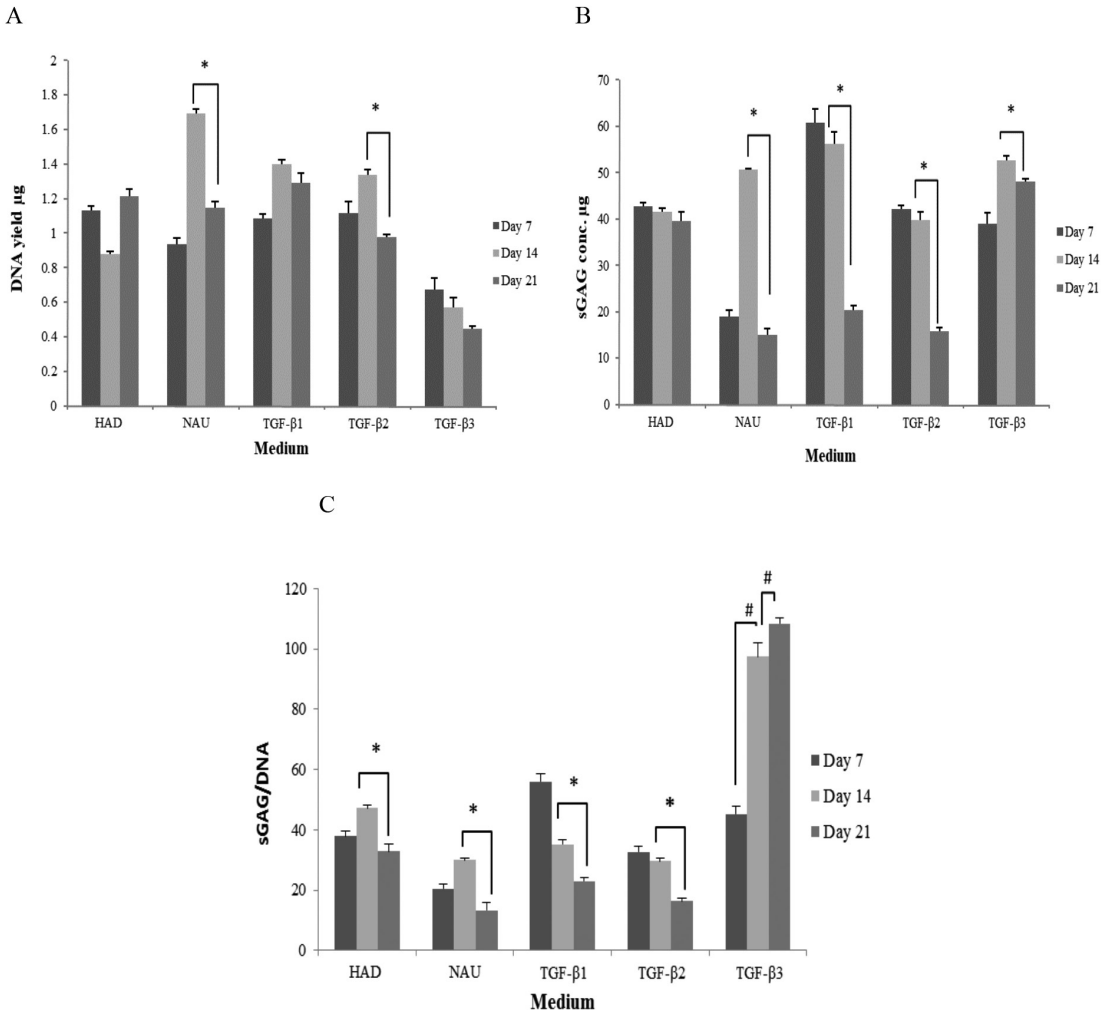

FIGURE 4. Biochemical analysis of the production of glycosaminoglycan phosphate (sGAG) for HADSC control (HAD), Human auricular chondrocyte control (NAU), HADSC induced with TGF-β1, TGF-β2 and TGF-β3. A) Total DNA content, B) Total GAGs content, and C) sGAG/DNA ratio. Symbol # denote significant increase on day 7, 14 and 21 cultures. Symbols * denote a significant decrease in day 14 and 21 cultures. * # $p<0.05$. The value showed mean ± SEM, $n=6$

21 of cultures. The highest gene expression also shown in HADSC induced with TGF-β3 on day 21 compared to other groups. The expression of Sox 9 gene (Figure 5(D)) was significantly ($p<0.05$) higher with 3.5 and 3.3 fold in the human chondrocyte control group and HADSC induced with TGF-β1 on day 21 compared to the day 14 of culture. The HADSC induced with TGF-β3 also exhibited a high gene expression with 1.6 fold on day 14 compared to day 7 of culture. In contrast, the expression gene of Type I collagen was significantly ($p<0.05$) reduced in expression by -19.0 and -36.0.0 folds in HADSC induced with TGF-β1 and TGF-β3 on day 21 compared to day 14. Meanwhile, other groups did not show any significant differences in the expression of Type I collagen between day 14 and 21 (Figure 5(E)). Expression on gene Type X collagen in HADSC control (HAD), human auricular chondrocyte control (NAU) and HADSC induced with TGF-β1 and TGF-β3 were showed significantly ($p<0.05$) decrease in gene expression by -1.0, -2.0, -1.8 and -2.5 folds on day 21 compared to day 14. Whereas, HADSC induced with TGF-β2 showed not significantly decrease in expression on day 21 (Figure 5(F)).

Our data showed that Type II collagen and core proteins were expressed HADSC induced by TGF-βs, and the expression of Sox 9 gene was occurred when the formation of aggregation. This consistent with the previous study that reported, Sox 9 gene expression during aggregation encourages the expression of Type II collagen gene and Aggrecan (Mueller & Tuan 2008). The expression of Aggrecan core protein was showed closely related to the production of extracellular matrix (ECM), especially glycosaminoglycan that shown in the previous sGAG analysis. However, the expression of fibroblastic gene (Type I collagen) and hypertrophic gene (Type X collagen) were seemed high in HADSC induced with TGF-βs. These finding suggested that the long induction time of stem cells to TGF-βs were affected the variety of features and hypertrophy of stem cells (Barry et al. 2001). This finding was supported by previous study that hMSC cultured in chondrogenic medium containing TGF-β3 was

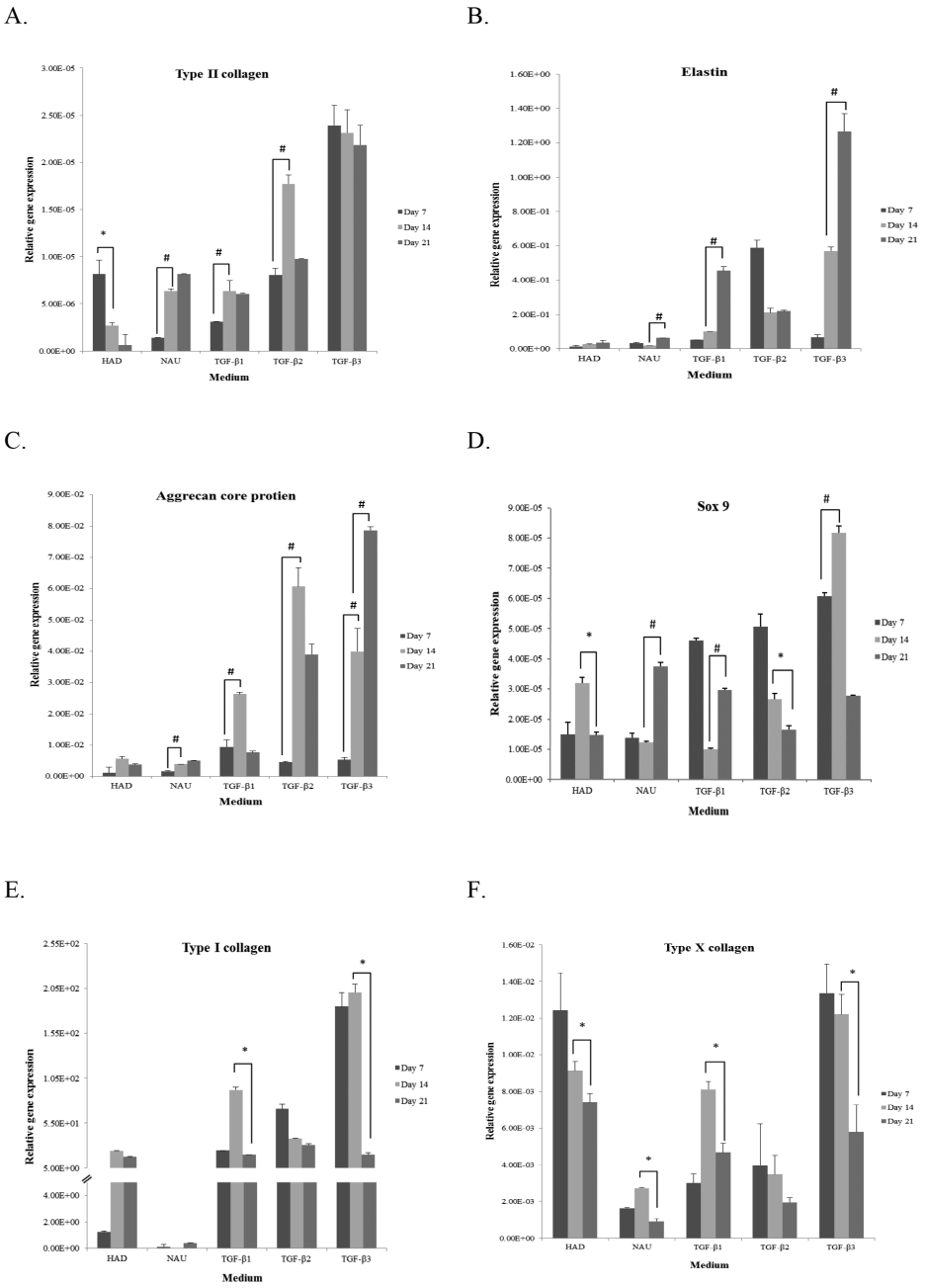

FIGURE 5. Relative gene expression: A) Type II collagen, B) Elastin, C) Aggrecan core protein, D) Sox 9, E) Type I collagen, and F) Type X collagen in HADSC control (HAD), Human auricular chondrocyte control (NAU), HADSC induced with TGF-β1, TGF-β2 and TGF-β3 on day 7, 14 and 21 cultures. Symbol # denote a significant increase between culturing days. Symbols * denote a significant decrease between culturing days. * # $p<0.05$. The value shows the mean ± SEM, $n = 6$

increased in the expression gene of Aggrecan core protein, Type II collagen and Type X collagen and consistently expressed in Type I collagen (Liao et al. 2015; Ude et al. 2017). Meanwhile, the expression of elastin gene was higher in HADSC induced with TGF-β3 suggested that the chondrogenic differentiation of stem cells was occurred more with TGF-β3 has potential to differentiate stem cells into auricular chondrocyte. The level expression of Elastin was suggested that the production of elastin protein requires a longer period of cultures. Previous studies also reported that the bone marrow stem required 28 day of induction to express the high expression gene of Elastin (Cai et al. 2015; Zhang et al. 2015).

CONCLUSION

In conclusion, TGF-β3 were found have better chondrogenesis effect in terms of cell regulation, proliferation/cell viability, chondrogenic differentiation and production of extracellular matrix protein (ECM) in induced HADSC. The members of transformation growth factor, TGF-β3 has showed a better chondrogenesis

process than TGF-β1 and TGF-β2 in the chondrogenic differentiation wherein cell aggregation, ECM production, especially glycosaminoglycan and collagen Type II and expression of chondrogenic markers genes such as Collagen Type II, Elastin, Aggrecan core protein and Sox 9. High expression gene of Elastin in HADSC induced TGF-β3 was suggested that TGF-β3 is better growth factor in HADSC differentiation into auricular chondrocyte compared to others. Additionally, the chondrogenic differentiation of HADSC by monocultures technique also exhibited dedifferentiation and hypertrophic differentiation characteristics whereas the expression of type I collagen and type X collagen were expressed during cell differentiation. Furthermore, the alternative culture technique need to explore to improve chondrogenic differentiation capacities of HADSC during culturing procedure for auricular reconstruction approach.

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