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Effects of culturing media on hepatocytes differentiation using Volvox sphere as co-culturing vehicle

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ABSTRACT

Volvox sphere is a unique design to mimic natural volvox consists of a large outer-sphere that contains smaller inner-spheres, which provide three-dimensional (3D) environment to culture cells. The purpose of this study is to co-culture mesenchymal stem cells (MSCs) and AML12 liver cells in Volvox spheres and to evaluate the effects of two media, DMEM and DMEM/F12 on the cultured cells. The results of this study shows that the 3D Volvox sphere can successfully be applied for co-culture of MSCs and AML12 liver cells, and the MSCs are able to differentiate into hepatocyte-like cells expressing hepatocyte-specific markers including albumin (ALB), alpha feto-protein (AFP) and cytokeratin 18 (CK18) mRNA expressions and producing CK18 and ALB proteins. Interestingly, the MSCs expressed higher ALB, AFP and CK18 mRNA expression at the initial 7-day culture by using DMEM, whereas, the MSCs expressed more mRNA expressions from 7-day to 14-day by the usage of DMEM/F12. The result demonstrated that DMEM and DMEM/F12 media could affect MSCs behaviors during a 14-day culture.

1. Introduction

The main functions of liver include maintenance of blood glucose level, metabolism of proteins and lipids, detoxification of urea, production of bile and participation in the endocrine function. Hepatocytes consist of the majority of liver mass and implement most functions of liver. These cells are physiologically mitosis-inactive but they exhibit considerable proliferative properties when the liver encounters damage. Culturing liver cells in a two-dimensional (2D) flask was difficult to preserve the functionality of liver, such as enzyme secretion. For hepatocytes, 2D culture *in vitro* conditions have been shown to quickly dedifferentiate by losing metabolic functions and structural integrity [1]. The absence of hepatic markers such as secretion of albumin in primary hepatocytes *in vitro* has been previously indicated [2]. Artificial liver systems combine the technology of bioreactor with cell immobilization, in order to mimic or to maintain the physiological function of a liver, so that it can be used to purify the blood of patients temporarily while waiting for a liver donor or regeneration of their own liver. Many studies were focused on optimizing the culturing environment of liver cells, including the usage of microspheres as a three dimensional (3D) culturing system [3,4]. The concept, techniques and devices for 3D hepatocyte culture were therefore investigated and developed. Different types of 3D hepatocyte cultures for high expression of liver-specific functions revealed in previous studies including sandwich, spheroid and gel entrapment cultures [5].

Mesenchymal stem cells (MSCs) have multiple potential of differentiation and high availability. Hence, MSCs provide an alternative cell source for replacement of primary hepatocytes in liver transplantation [6]. MSCs derived from bone marrow, umbilical cord blood or adipose tissue possesses potential to differentiate into hepatocytes in rats, mice and humans under specified *in vitro* growth conditions [7]. Bone marrow and apidose tissue have been shown as the two main sources for isolation of mesenchymal stem cells. Also, MSCs exhibit long periods of time in culture without losing differentiation capacity [8]. It has been found that rat MSCs co-cultured with primary hepatocytes maintained normal proliferation and viability and significantly expressed mRNA for ALB, AFP

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and CK-18 [9]. Another cell type of liver, hepatic stellate cells were also able to induce the differentiation of MSCs into hepatocyte-like cells shown by expression of ALB, AFP, CK-18, glutamine synthetase and phosphoenolpyruvate carboxykinase as well as detection of glycogen deposition [10]. These studies demonstrated that liver cells are able to induce the differentiation of MSCs into hepatocytes-like cells or hepatocytes *in vitro*.

Volvox sphere is a bio-mimicking concept of biomaterial structure design that consists of a large sphere containing smaller microspheres, which is able to encapsulate chemicals, drugs and/or cells [11]. This unique spherical device is prepared via a high voltage electrostatic field system (HVEFS) using natural polymer alginate and collagen. In the current study, Volvox spheres were employed as co-culturing vehicle by encapsulating MSCs and AML12 liver cells. However, the issue regarding to the culture medium arose when DMEM was originally used as basal DMEM medium for MSCs while DMEM/F12 medium was for AML12 cells [12,13]. Once the two types of cells were encapsulated together, the selection of culturing media became a crucial concern. Therefore, the effects of the two media, DMEM and DMEM/F12, on the fates of the MSCs co-cultured with AML 12 liver cells in Volvox sphere were evaluated by live/dead staining, real-time PCR and flow cytometry analyses.

2. Materials and methods

2.1. Materials

Alginate (1.5×10^5 Da) and poly-L-lysine (PLL, 1.5×10^4 Da) were purchased from Sigma (St. Louis, MO, USA). Collagen was purchased from Advanced Biomatrix (San Diego, CA). Calcium chloride was obtained from J.T. Baker (Japan). Dulbecco's Modified Eagle's Medium (DMEM) and Dulbecco's Modified Eagle's Medium: Nutrient Mixture F-12 (1: 1) (DMEM/F12) were purchased from GIBCO (U.S.A.). All chemicals used in this study were of reagent grade.

2.2. MSCs isolation and cells culture

Bone marrow was aspirated and collected from the femur bones of 3-week-old Sprague–Dawley rats. The bone marrow was diluted with 4 mL of DMEM and then centrifuged for 15 min. The enriched cells were cultured in a low-glucose DMEM supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2. After 3 days, non-adherent hematopoietic cells were discarded and the adherent cells were washed with phosphate buffered solution (PBS). The DMEM medium was replenished every 3 days. When the cells reached approximately 90% confluence, then they were trypsinized for passage with 0.25% trypsin/EDTA. The second passage MSCs was used through all experiments. AML12 liver cells were cultured in DMEM/F12 medium.

2.3. Preparation of cell-encapsulated Volvox spheres

The cell-encapsulated Volvox spheres was prepared according to our previous published work [11]. Briefly, a final concentration of 1×10^7 cells/mL AML12 cells was mixed into sterilized alginate solution (1 mL), and extruded into 100 mM calcium chloride via high HVEFS to prepare the cell-encapsulated inner spheres. These produced spheres were treated with 0.05% (w/v) PLL for 5 min and washed carefully with PBS. These spheres were mixed with 1×10^7 cells/mL of MSCs in 1 mL alginate solution and a larger diameter needle was used to produce the cell-encapsulated Volvox spheres.

2.4. Live/dead cells staining

The viability of MSCs and AML 12 cells encapsulated inside the Volvox spheres was examined with a live/dead cell assay (Invitrogen, UK). 1 mL of PBS solution containing 2.5 μL/mL of 4 μM ethidium homodimer-1 (EthD-1) assay solution and 1 μL/mL of 2 μM calcein AM solution was prepared. Then, 100 μL of this assay solution was added to the culture, and the mixture was placed in a 37 °C incubator with a humidified atmosphere and 5% CO_2. The solution was removed after 15 min and the sample was viewed under a fluorescence microscope with 494-nm (green, calcein) and 528-nm (red, EthD-1) excitation filters (Olympus IX71, Japan).

2.5. Real time RT-PCR analysis

Fifty Volvox spheres were collected and then treated with 50 mM sodium citrate to dissolve the outer sphere and collect the encapsulated MSCs. The inner-spheres that encapsulated AML 12 cells remained intact because they were treated with 0.05% (w/v) PLL and could be filtered out through a cell strainer (BD Falcon, USA). Total MSCs RNA was extracted using an RNA extraction kit (Favorgen, Taiwan) according to the manufacturer instructions. The extracted RNA was reversely transcribed into cDNA using an iScript™ cDNA synthesis kit (BioRAD, USA), followed by amplification of cDNA using albumin (ALB), alpha-fetoprotein (AFP) and cytokeratin 18 (CK18) primers on a StepOne™ real time PCR (Life technologies, USA). The level of each PCR value was normalized to the level of housekeeping gene ARBP. The relative quantification (RQ) of specific gene expression was determined using the $2^{-\triangle\triangle Ct}$ method [14,15].

2.6. Flow cytometry analysis

Twenty Volvox spheres were collected and treated to collect the encapsulated MSCs. The MSCs were treated with 4% paraformaldehyde for 10 min, permeabilized by 0.1% triton X for 20 min, and then blocked by 5% bovine serum albumin for 30 min. The cells were incubated with mouse anti-CK18 primary antibody (Abcam, England) for 30 min, and FITC-conjugated goat anti-mouse secondary antibody (Santa Cruz Biotech, USA) for another 30 min. The prepared sample was subjected to flow cytometry analysis using a flow cytometer (BD Accuri C6, USA).

2.7. Albumin production analysis

The albumin secreted from the Volvox spheres-encapsulated cells was measured by using an ELISA 96-well quantitative rat albumin core kit (Koma biotech, Korea). The absorbance of each well was read on a spectrophotometer (Multiskan Co, Thermo Scientific, USA) at 450 nm.

2.8. Statistics

All data are expressed as mean ± standard deviation (SD). Data were statistically analyzed by independent sample *t*-test or ANOVA. Values of $p < 0.05$ were considered to be statistically significant.

3. Results

3.1. Production of cell-encapsulated Volvox spheres

Fig. 1(A–C) shows the schematic diagram for the production of cell-encapsulated Volvox spheres. These Volvox spheres were divided into two groups, one group was cultured with DMEM medium and the other group was cultured with DMEM/F 12

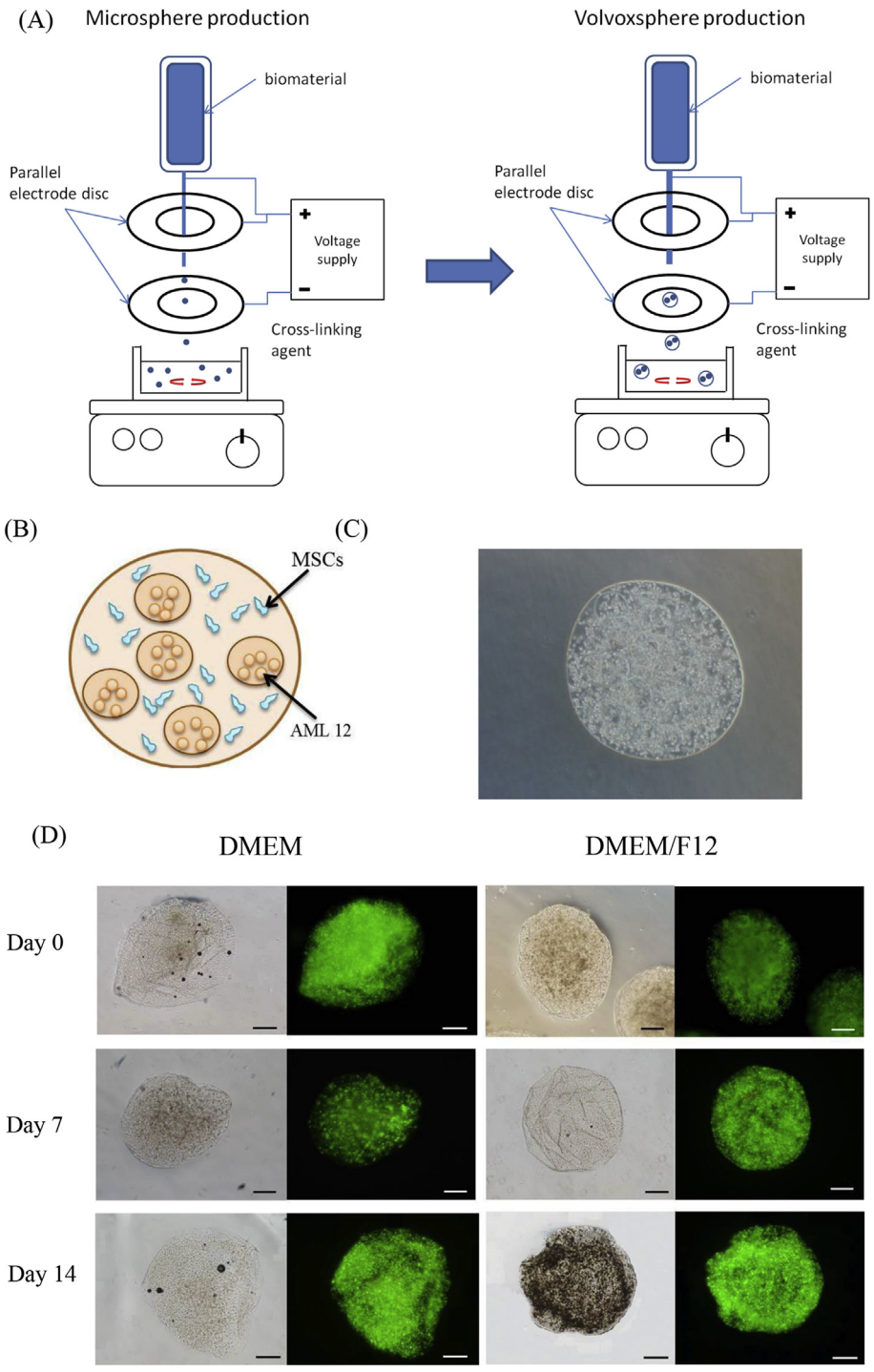

Fig. 1. (A) Schematic diagram of the production of cell-encapsulated Volvox spheres. (B) A drawing of a cell-encapsulating Volvox sphere and (C) a produced cell-encapsulating Volvox sphere. (D) Live/dead staining of encapsulated cells at 0, 7 and 14 days post-encapsulation in Volvox spheres. Live cells are stained with green color. (Scale bar = 200 μm). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

medium. After a 7-d and 14-d incubation, the MSCs encapsulated in Volvox spheres were draw out for further differentiation analyses.

3.2. Live/dead staining of encapsulated cells

The viability of encapsulated cells were determined using live/dead staining where green fluorescence representing live cell while red fluorescence representing dead cell. Fig. 1(D) shows the staining result of the two experimental groups with an incubation period of 0, 7 and 14 days, in which a higher number of live cells was observed in both DMEMD and DMEM/F12 groups at 14 day incubation as compared to the 7 day culture. These findings might be related to the micro-environment of MSCs where these cells require certain period of time to adapt to the new environment of the alginate and collagen in Volvox spheres.

3.3. mRNA expression of ALB, AFP and CK18 in encapsulated cells

The differentiation of MSCs began as soon as the initiation of the co-culture process. mRNA expression of ALB was elevated in both DMEM and DMEM/F12 groups but at different pace. For the DMEM group (Fig. 2(A)), a continuous increasing trend started from a 1.8 fold enhancement on day 7, and then 11.4 fold up-regulation on day 14 was observed whereas in the DMEM/F12 group ALB mRNA was increased by a 5.5 fold on day 7 and a 5.4 fold elevation was observed on day 14. On the other hand, both groups showed decreased mRNA expression of AFP on day 7, but on day 14, a 6.5 fold increase was observed in the group of DMEM/F12 (Fig. 2(B)). As for CK18 (Fig. 2(C)), both DMEM and DMEM/F12 groups exhibited increases in mRNA expression on day 7 (28.6 fold and 60.7 fold, respectively) but on day 14 the enhancement in CK18 mRNA returned to a 9.3 fold and a 41.6 fold, respectively.

3.4. Flow cytometric analysis for CK18 protein in encapsulated cells

The data of flow cytometric analysis showed a significant increase (19.4%) of CK18 protein within 7 days in the DMEM group. The incubation of DMEM/F12 resulted in a significant increase (24.8%) between day 7 and day 14 (Fig. 3).

3.5. ALB secretion by the encapsulated cells in Volvox spheres

The ALB secretion of Volvox sphere encapsulated cells was shown in Fig. 4. AML12 cells alone secreted approximately 40 ng ALB after a 14-day culture. In contrast, the encapsulated AML 12 cells and MSCs in the Volvox spheres exhibited over 3-fold enhancement of ALB secretion, approximately 140 ng and 120 ng for the DMEM and DMEM/F12, respectively.

4. Discussion

Liver transplantation is the gold standard for the treatment of end-stage liver disease. Approximately 35% of European patients on the waiting list do not receive the required organ due to general shortage of donor organs, while the percentage appears to be even higher in USA. Therefore, transplantation of hepatocytes seems to be a potential choice for patients who need whole liver transplantation [7]. Evidence has shown that MSCs are a valuable source for liver repair and regeneration in preimmune fatal sheep [16]. The *in vitro* differentiation potential of MSCs from bone marrow or adipose tissues has been verified in rodent and human models [8]. The hepatectomized SCID mouse expressed hepatocyte markers such as albumin and CK18 after bone marrow-derived MSCs pre-differentiated into hepatocyte-like cells *in vitro* were xenografted to mouse livers [17,18]. Hepatocyte-differentiated MSCs function within the host liver at significant higher rates although both undifferentiated and hepatocyte-differentiated MSCs integrations are functional [6]. Importantly, 2D co-cultures of MSCs with hepatocytes have been shown to induce better hepatic differentiation efficiency than treatment with hepatocyte growth factor [8], demonstrating that hepatocytes are capable of inducing MSCs differentiation into hepatocytes-like cells.

At the retro-differentiation stage, AFP mRNA expression of the DMEM/F12 group elevated by over 6.5-fold at day 14 compared with day 0 whereas the counterpart of the DMEM group seemed unchanged, suggesting more abundant nutritional components in DMEM/F12, such as Biotin, Vitamin B12, Cu^{2+}, Fe^{2+}, Zn^{2+} and thymidine which are necessary for the processes of retro-differentiation of hepatocytes/hepatocytes-like cells, or at later phase (day 7–14 in this study). When focused on the liver marker CK18 mRNA expression, DMEM/F12 with rich nutritional

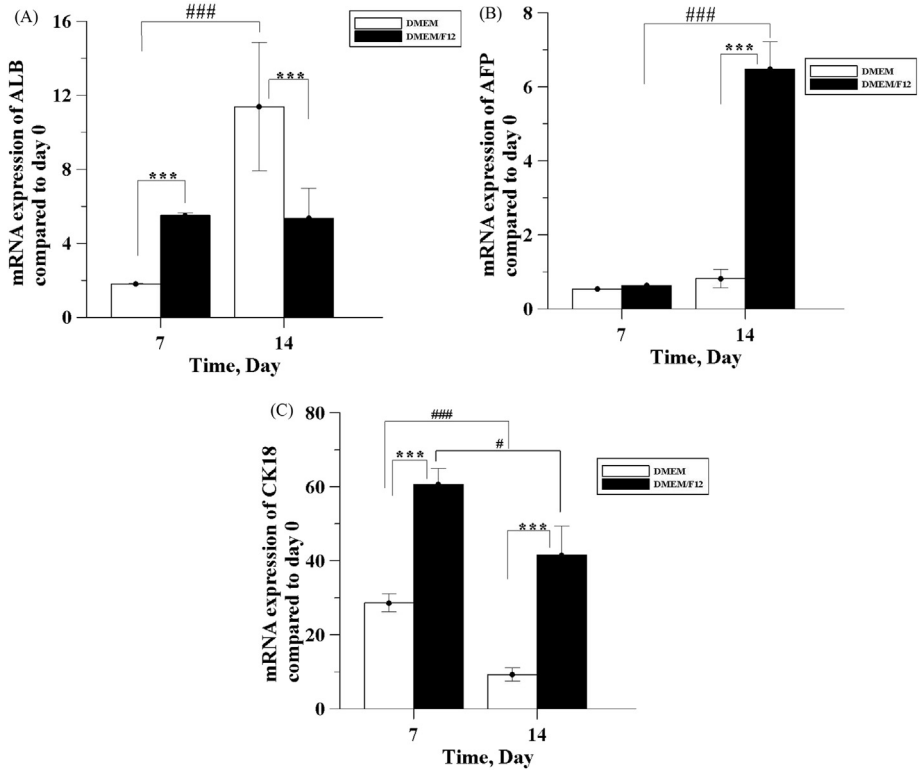

Fig. 2. Real time RT-PCR analyses in mRNA expression of (A) ALB, (B) AFP and (C) CK18 on day 7 and day 14 compared with those on day 0. (The significant differences are based on comparisons with the different medium on same incubation period (*) or with the different incubation period on same medium (#): *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, #$p < 0.05$, ##$p < 0.01$, and ###$p < 0.001$, *$p < 0.001$).

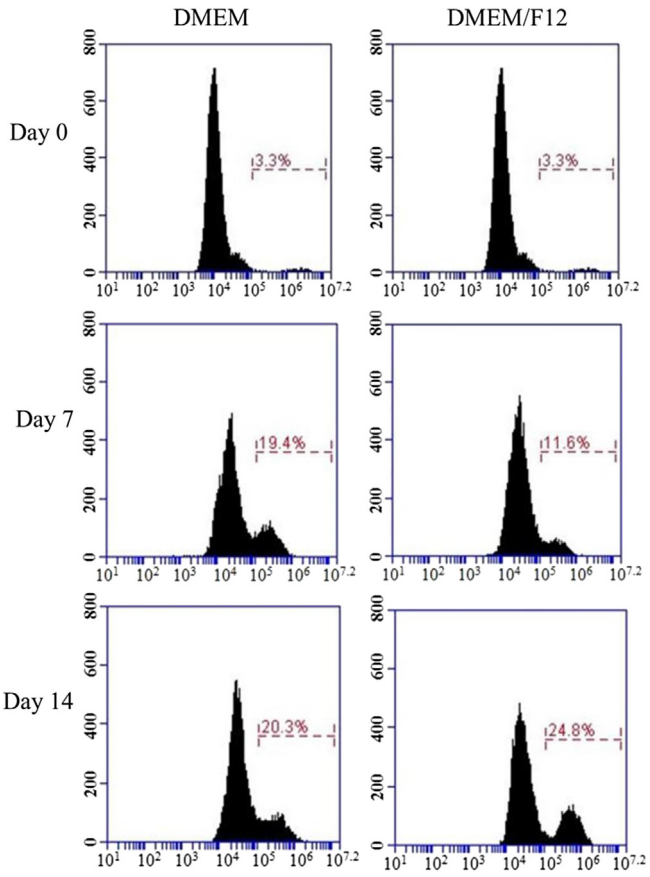

Fig. 3. Flow cytometry analyses demonstrating that 19.4% and 20.3% of MSCs expressed CK18 when cultured in DMEM after 7-day and 14-day incubation where 11.6% and 24.8% of MSCs expressed CK18 when cultured in DMEM/F12.

substances may also be a more suitable medium for differentiation of MSCs into hepatocytes/hepatocytes-like cells in the Volvox spheres. Nonetheless, flow cytometric data still displayed a significant increase in CK18 protein within day 7 as well as between day 7 and 14 in DMEM as in DMEM/F12. On the other hand, the various nutritional components in DMEM/F12 seem not that essential for the secretion and mRNA expression of albumin for the differentiation of MSCs into hepatocytes/hepatocytes-like cells in the Volvox spheres. Although mRNA for ALB in DMEM was increased for DMEM/F12, no significant difference in ALB secretion between DMEM and DMEM/F12 groups within 14 days was observed (Fig. 2(A)). Combining the data described above, it can be concluded that DMEM might be the proper medium for the co-culture of MSCs with AML12 cells but after day 7, DMEM/12, which is a richer nutritional supplement, should be the appropriate medium for MSCs/AML12 cells co-cultured system in the Volvox spheres. This is also consistent with the fact that the DMEM/F12 medium is usually used for the hepatocyte cell line AML12 to maintain the metabolic functions of the liver cells.

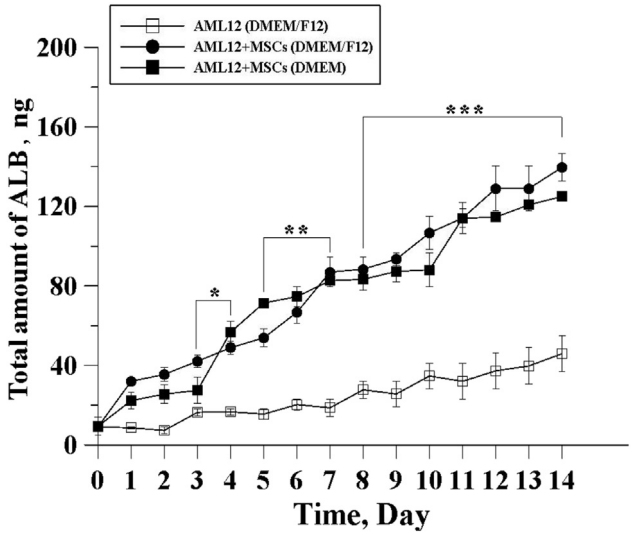

Fig. 4. Albumin secretion of Volvox sphere encapsulated cells. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, comparing the differences between AML12 and AML12 with MSCs at 1-day interval by independent sample t-test).

The reestablishment of AFP mRNA expression on day 14 in the current study could be associated with a phenomenon known as retro-differentiation [19], which occurs at the cell repair phase when cells encounter stress such as limited nutrient availability or accumulated waste products. On the other hand, high glucose has been shown to favor osteogenic differentiation from human MSCs [20], which is similar with the finding that DMEM seems the appropriate medium for the co-culture in the current study before day 7, particularly in the early 3 days. However, high glucose has been found to induce proteome changes and may attenuate antioxidant capacity in hepatocytes [21,22]. These previous findings might be partially associated with attenuated increases in AFP and CK18 mRNA expression in DMEM and with that both DMEM and DMEM/F12 medium cultures encountered decreased viability of hepatocytes/hepatocytes-like cells in this present study (data not shown here). The albumin (ALB) secretion of Volvox sphere encapsulated cells was shown in Fig. 4. As shown, AML12 cells alone secreted approximately 40 ng ALB after a 14-day culture. However, the encapsulated AML 12 cells and MSCs in the Volvox spheres displayed an over 3-fold increased ALB secretion, approximately 140 ng and 120 ng, for the DMEM and DMEM/F12 respectively. The increased ALB secretion in the group of MSCs co-cultured with AML12 cells compared with that produced in the AML 12 cells alone group indicated that the encapsulated MSCs have differentiated into hepatocytes/hepatocytes-like cells and performed the hepatic specific function of ALB secretion. Based on the obtained results, we suggest that the co-culture of MSCs with hepatocytes not only is able to induce differentiation of MSCs into hepatocytes-like cells/hepatocytes but also can improve the preservation of metabolic functions for hepatocytes. Despite of these merits, live/dead cell staining results showed that the viability of MSCs co-cultured with AML12 cells in both DMEM and DMEM/F12 at day 7 and 14 was mildly to moderately reduced, compared with that at day 0, implicating some specific growth factors for hepatocyte growth or better micro-circulation of medium may be needed in the Volvox sphere culture vehicle.

According to the obtained results in the current study, the majority of the encapsulated MSCs was remained alive and had undergone hepatic differentiation, indicating that the 3D co-culture of MSCs with hepatocyte cell line in Volvox spheres is feasible for hepatic cell differentiation from MSCs. The type of culturing media indeed affected the efficiency of hepatic differentiation. Therefore, several potential techniques could to improve the cell viability in future. These include changing the basal medium at different stages of the culturing process, such as use of DMEM during the first 7 days and replacement with DMEM/F12 in the following 7 days. In addition, application of specific growth factors and/or possible improvement of micro-circulation within the Volvox sphere are the alternatives to further enhance the cell viability of the differentiated cells in the Volvox spheres.

Conflict of interest

The authors have no conflict of interest.

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Transparency document

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References

[1] C.W. Lan, I. Manousakas, S.J. Chang, et al., Influences of nano-sized type I collagen molecules on hepatocyte cultures, J. Med. Biol. Eng. 29 (2) (2009) 68–74.
[2] Z.F. Van, W. Mikulits, Hepatospheres: three dimensional cell cultures resemble physiological conditions of the liver, World J. Hepatol. 2 (1) (2010) 1–7.
[3] M. Ringel, M.A von Mach, R. Santos, et al., Hepatocytes cultured in alginate microspheres: an optimized technique to study enzyme induction, Toxicology 206 (1) (2005) 153–167.
[4] N.E. Simpson, S.C. Grant, L. Gustavsson, V.-M. Peltonen, S.J. Blackband, Biochemical consequences of alginate encapsulation: a NMR study of insulin-secreting cells, Biomaterials 27 (12) (2006) 2577–2586.
[5] Q. Meng, Three-dimensional culture of hepatocytes for prediction of drug-induced hepatotoxicity, Expert. Opin. Drug Metab. Toxicol. 6 (6) (2010) 733–746.
[6] B. Christ, P. Stock, Mesenchymal stem cell-derived hepatocytes for functional liver replacement, Front Immunol. 3 (2012) 168.
[7] P. Stock, S. Brückner, S. Ebensing, et al., The generation of hepatocytes from mesenchymal stem cells and engraftment into murine liver, Nat. Protoc. 5 (4) (2010) 617–627.
[8] M.A. Puglisi, N. Saulnier, A.C. Piscaglia, et al., Adipose tissue-derived mesenchymal stem cells and hepatic differentiation: old concepts and future perspectives, Eur. Rev. Med. Pharmacol. Sci. 15 (2011) 355–364.
[9] Z. Qihao, C. Xigu, C. Guanghui, Z. Weiwei, Spheroid formation and differentiation into hepatocyte-like cells of rat mesenchymal stem cell induced by co-culture with liver cells, DNA Cell Biol. 26 (7) (2007) 497–503.
[10] X. Deng, Y.X. Chen, X. Zhang, et al., Hepatic stellate cells modulate the differentiation of bone marrow mesenchymal stem cells into hepatocyte-like cells, J. Cell. Physiol. 217 (1) (2008) 138–144.
[11] B. Teong, S.J. Chang, C.W. Chuang, S.M. Kuo, I. Manousakas, The production of volvox spheres and their potential application in multi-drugs encapsulation and release, Mater. Sci. Eng. C 33 (8) (2013) 4859–4866.
[12] R.H. Pierce, J.S. Campbell, A.B. Stephenson, et al., Disruption of redox homeostasis in tumor necrosis factor-induced apoptosis in a murine hepatocyte cell line, Am. J. Pathol. 157 (1) (2000) 221–236.
[13] Z. Ke, X. Mao, S. Li, Dynamic expression characteristics of Notch signal in bone marrow-derived mesenchymal stem cells during the process of differentiation into hepatocytes, Tissue Cell 45 (2) (2013) 95–100.
[14] M.W. Pfaffl, A new mathematical model for relative quantification in real-time RT–PCR, Nucleic Acids Res. 29 (9) (2001) 2003–2007.
[15] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative and the 2(-delta delta C(T)) method, Methods Archiv. Exp. Pathol. 25 (2001) 402–408.
[16] J. Chamerlain, T. Yamagami, E. Colletti, et al., Efficient generation of human hepatocytes by the intrahepatic delivery of clonal human mesenchymal stem cells in fetal sheep, Hepatology 46 (2007) 1935–1945.
[17] P.A. Lysy, D. Campard, F. Smets, et al., Persistence of a chimerical phenotype after hepatocyte differentiation of human bone marrow mesenchymal stem cells, Cell Prolif. 41 (1) (2008) 36–58.
[18] H. Aurich, M. Sgodda, P. Kaltwasser, et al., Hepatocyte differentiation of mesenchymal stem cells from human adipose tissue in vitro promotes hepatic integration in vivo, Gut 58 (4) (2009) 570–581.
[19] W.D. Kuhlmann, P. Peschke, Hepatic progenitor cells, stem cells, and AFP expression in models of liver injury, Int J Exp Pathol. 87 (5) (2006) 343–359.
[20] Y.M. Li, T. Schilling, P. Benisch, et al., Effects of high glucose on mesenchymal stem cell proliferation and differentiation, Biochem. Biophys. Res. Commun. 363 (1) (2007) 209–215.
[21] J.Y. Chen, H.C. Chou, Y.H. Chen, H.L. Chan, High glucose-induced proteome alterations in hepatocytes and its possible relevance to diabetic liver disease, J. Nutr. Biochem. 24 (11) (2013) 1889–1910.
[22] Y. Ikeda, T. Suehiro, K. Arii, Y. Kumon, K. Hashimoto, High glucose induces transactivation of the human paraoxonase 1 gene in hepatocytes, Metabolism 57 (12) (2008) 1725–1732.