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Novel *In Vitro* Culture Condition Improves the Stemness of Human Dermal Stem/Progenitor Cells

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Cell therapy using adult stem cells has emerged as a potentially new approach for the treatment of various diseases. Therefore, it is an essential procedure to maintain the stemness of adult stem cells for clinical treatment. We previously reported that human dermal stem/progenitor cells (hDSPCs) can be enriched using collagen type IV. However, hDSPCs gradually lose their stem cell properties as *in vitro* passages continue. In the present study, we developed optimized *in vitro* culture condition to improve the stemness of these hDSPCs. To evaluate whether the stemness of hDSPCs is well sustained in various culture conditions, we measured the expression levels of SOX2, NANOG, and S100B, which are well-known representative dermal progenitor markers. We observed that hDSPCs grown in three-dimensional (3D) culture condition had higher expression levels of those markers compared with hDSPCs grown in two-dimensional (2D) culture condition. Under the 3D culture condition, we further demonstrated that a high glucose (4.5 g/L) concentration enhanced the expression levels of the dermal progenitor markers, whereas O_2 concentration did not affect. We also found that skin-derived precursor (SKP) culture medium was the most effective, among various culture media, in increasing the dermal progenitor marker expression. We finally demonstrated that this optimized culture condition enhanced the expression level of human telomerase reverse transcriptase (hTERT), the proliferation, and the multipotency of hDSPCs, an important characteristic of stem cells. Taken together, these results suggested that this novel *in vitro* culture condition improves the stemness of hDSPCs.

INTRODUCTION

Adult stem cells are indispensable for maintaining the homeostasis of all of the tissues in our body. These cells have been identified in various tissues of the body, including bone marrow (Anderson et al., 2001; Jiang et al., 2002a; Pereira et al., 1995; Pittenger et al., 1999), brain, skeletal muscle (Jiang et al., 2002b), heart (Yoon et al., 2007) and adipose tissue (Zuk et al., 2001; 2002). These cells can differentiate into various cell types and have the characteristic of self-renewal.

Skin, the largest tissue in our body, is considered to be an attractive resource for isolating stem cells owing to its easy accessibility. Adult stem cells have also been found in skin dermis (Amoh et al., 2005; Biernaskie et al., 2009; Blanpain et al., 2006; Driskell et al., 2009; Fernandes et al 2004; Fuchs, 2009; Ohyama et al., 2006; Shim et al., 2012; 2013; Toma et al., 2001; 2005). Although some scientists have tried to search for universal dermal stem cell markers on the plasma membrane for easy isolation, specific markers have still not been identified. On the contrary, a few intracellular specific markers are identified in dermal stem cells. SOX2, a SRY transcription factor, plays an essential role in maintaining stemness in embryonic stem cells and some adult stem cells such as the dermal papilla cells (Rendl et al., 2005; Wegner and Stolt, 2005). NANOG, another embryonic stem cell marker, is also expressed in adult mesenchymal stem cell populations derived from bone marrow, adipose tissue, and dermis (Hill et al., 2012; Riekstina et al., 2009; Szade et al., 2011). S100B are known to be specifically upregulated in dermal stem cells (Driskell et al., 2009).

We have previously reported that collagen type IV-enriched human dermal stem/progenitor cells (hDSPCs) show stem cell-like characteristics (Shim et al., 2012). Compared to other somatic cells, hDSPCs have a relatively high proliferation property and show higher expression levels of SOX2 and S100B. They can also differentiate into mesodermal cells, such as adipocytes, osteoblasts, chondrocytes, and ectodermal progeny, such as neuronal cells. Thus, hDSPCs can serve as a good model for studying the regenerative capability of all tissues, which can aid in the development of human cell therapies.

Generally, all primary cells, including adult stem cells, undergo only a limited number of cell divisions under standard culture conditions, due to a phenomenon called cellular senescence and adult stem cells lose their stemness during long-term culture. It is also difficult to obtain adult stem cells because of their limited quantities in the tissue. According to some previous literatures, they are considered to be composed of 0.01% to 0.001% of the total cell number (Hill et al., 2012; Rendl et al., 2005; Riekstina et al., 2009; Shim et al., 2013; Wegner and Stolt, 2005; Wu et al., 2010). We found that hDSPCs in small quantity gradually lose pluripotency and the ability to proliferate once they are grown over long-term culture. Thus, the development of optimized *in vitro* culture conditions is necessary for

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studying and using these hDSPCs.

In the present study, we investigated which *in vitro* culture conditions are appropriate for maintaining the stemness of hDSPCs by measuring the expression levels of dermal progenitor markers such as SOX2, NANOG and S100B. We found that the specific markers of hDSPCs are better preserved in suspension culture conditions than in two-dimensional (2D) culture conditions. Under the 3D condition, we observed that a high glucose concentration (4.5 g/L), but not hypoxic condition, induced the expression of the specific markers. We also demonstrated that skin precursor (SKP) culture medium contributed to maintain the stemness of hDSPCs relatively well among various culture media. We showed that SKP medium containing high-glucose DMEM under normoxic 3D culture condition enhanced the expression level of human telomerase reverse transcriptase (hTERT) in hDSPCs compared to that of freshly enriched hDSPCs by collagen type IV. We finally confirmed that this novel culture condition improved both the proliferative property and the multipotency of hDSPCs.

MATERIALS AND METHODS

Chemicals and materials

The growth factors, fibroblast growth factor 2 (FGF2) and epidermal growth factor (EGF) were purchased from R&D Systems (USA). The cell culture media including F12, B27 supplement, and AmnioMAX C-100 complete medium were obtained from Invitrogen (USA). Hair follicle dermal papilla cell (HFDPC) growth medium was purchased from Cell Applications (USA). Normal human dermal fibroblasts (NHDFs), fetal bovine serum (FBS) and Dulbecco's modified Eagle medium (DMEM) were bought from Lonza (Switzerland).

All other chemicals were purchased from Sigma-Aldrich (USA), and sterile plastic materials were obtained from BD biosciences (USA) or Nunc (UK).

Cell culture and enrichment of hDSPCs

NHDFs were cultured in DMEM containing 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin at 37°C in 5% CO_2. NHDFs were used for the experiments within three passages.

We previously demonstrated that hDSPCs can be enriched using collagen type IV (Shim et al., 2012). Briefly, when NHDFs were plated onto collagen type IV-coated culture dishes, a portion of the population adhered to the culture dish. Cells adhering within 5 min (hDSPCs) or between 5 min and 12 h (non-hDSPCs) were separated and transferred to different culture dishes.

Adherent culture of hDSPCs

hDSPCs were centrifuged for 5 min at 1,000 × *g*. The pellet was resuspended in DMEM, 100 U/ml penicillin and 100 μg/ml streptomycin and dissociated on the (i) gelatin (1 mg/ml)-coated; (ii) collagen type I (200 μg/ml)-coated; and (iii) collagen type IV (200 μg/ml)-coated tissue culture dishes, respectively.

Sphere formation of hDSPCs

For suspension cultures, collagen type IV-enriched hDSPCs were plated in HydroCell plates (Nunc) with SKP medium (Biernaskie et al., 2009; Toma et al., 2001; 2005) or 2-hydroxyethyl methacrylate (poly-HEMA) as described previously (Kuroda et al., 2010). Briefly, 15 mg of poly-HEMA was dissolved in 1 ml of 95% ethanol by shaking at 37°C; the culture dish was coated with this solution and then air-dried on the clean bench overnight.

Morphology analysis

Cell morphology and histochemistry were analyzed at 100X or 200X magnification on a phase-contrast microscopy (IX71; Olympus, USA). Images were taken with an Olympus camera (DP70).

RNA isolation and real-time quantitative RT-PCR

Total RNA was extracted using TRIzol™ (Invitrogen) according to the manufacturer's instructions. cDNA was generated using the First Strand cDNA Synthesis Kit (Toyobo, Japan). Real-time quantitative RT-PCR was performed with TaqMan® universal II PCR Master Mix and results were analyzed using 7500 Fast Real-Time PCR System (Applied Biosystems, USA). The reaction was run at 50°C for 20 s and 95°C for 10 min followed by 45 cycles at 95°C for 15 s and 60°C for 1 min and then one cycle at 95°C for 1 min. TaqMan® Gene Expression Assays were obtained from Applied Biosystems. cDNA samples were analyzed for the following genes: SOX2 (Hs01053049_s1); S100B (Hs00389217_m1); NANOG (Hs04260366_g1); hTERT (Hs00972656_m1); osteoglycin, Hs00247901_m1; osteocalcin, Hs01587814_g1; peroxisome proliferator-activated receptor gamma (PPARγ), Hs01115513_m1; leptin , Hs00174877_m1; adiponectin, Hs00605917_m1; fatty acid binding protein 4 (FABP4). Quantification was obtained by normalizing the target genes against the GAPDH housekeeping gene. Data were analyzed using the Comparative C_T method.

Multilineage differentiation of hDSPCs

Collagen type IV-enriched hDSPCs were analyzed for their capacity to differentiate into adipogenic and osteogenic lineages as described previously (Shim et al., 2012; 2013).

Adipogenic differentiation was induced in adipogenic medium (1 g/L glucose-DMEM supplemented with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, 0.5 mM IBMX, 1 μM dexamethasone, 10 μg/ml insulin and 2 μM troglitazone (Sigma) for 10 days. To induce osteogenic differentiation, hDSPCs were treated with the hMSC Osteogenic BulletKit (Lonza) for 2 weeks.

The differentiation medium was changed every 2-3 days.

Histochemical analyses

To examine the presence of intracellular lipid droplets in differentiated adipocytes, Oil Red O staining was performed. The cells were fixed with 4% paraformaldehyde for 10 min at RT and then washed with 60% isopropanol. Then the cells were treated with Oil Red O staining solution (Sigma) at RT for 10 min. Excess staining was removed with 70% ethanol followed by wash with PBS. The Oil Red O contents were extracted with 100% isopropanol and quantified with a SpectraMax 190 microplate reader at 500 nm.

Alkaline phosphatase (ALP) staining was performed to examine osteogenic differentiation (Takara, Japan). Differentiated cells were fixed with citrate buffer (pH 5.4) containing 45% acetone and 10% methanol for 5 min at RT. Then the cells were incubated with ALP substrate for 30 min at 37°C and washed three times with double-distilled water.

Statistical analyses

Statistical analyses were performed using Student's *t*-test with the MINITAB software (Minitab Inc., USA). The results are expressed as the mean ± standard deviation of at least three independent experiments.

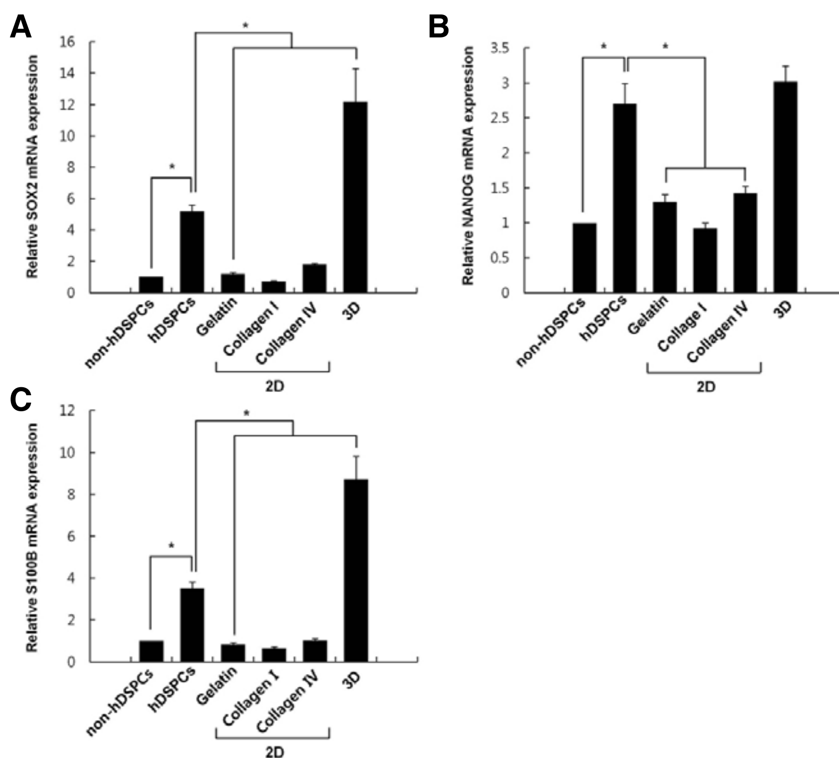

Fig. 1. Effects of suspension culture condition on hDSPCs. NHDFs were plated onto collagen type IV-coated culture dishes. Cells adhering within 5 min (hDSPCs) or between 5 min and 12 h (non-hDSPCs) were separated and transferred to different culture dishes. Collagen type IV-enriched hDSPCs were cultured on gelatin, collagen type I, or collagen type IV-coated tissue culture dishes or HydroCell plates for suspension culture during 48 h. Total RNA was extracted, and real-time quantitative RT-PCR was performed to measure SOX2 (A), NANOG (B) and S100B (C). Values represent the mean expression ± standard deviation (S.D.) of three independent experiments. $^*p < 0.01$.

RESULTS

Effects of matrix environments on the stemness of hDSPCs

We previously demonstrated that hDSPCs can be enriched using collagen type IV (Shim et al., 2012). After NHDFs are plated onto culture dishes coated with collagen type IV, a small population adheres to the culture dish quickly, while the rest of the cells take more time to adhere. In particular, 1-5% of cells adhere within 5 min, which are classified as hDSPCs, whereas the remaining cells adhere between 5 min and 12 h, and are classified as non-hDSPCs. The different type of cells are separated and transferred to different culture dishes. Although we demonstrated that the hDSPCs shows both multipotency and higher proliferative ability, similar to the characteristics of other adult stem cells, we also found that the hDSPCs gradually lose their stemness with consecutive *in vitro* culture on 2D culture dishes.

Thus, we first evaluated whether extracellular matrix materials such as gelatin, collagen type I, and collagen type IV or suspension culture condition in serum-free DMEM have an effect on the stemness of hDSPCs. Real-time quantitative RT-PCR revealed that monolayer cultures with gelatin, collagen type I and collagen type IV reduced the mRNA levels of dermal progenitor cell markers, SOX2, NANOG, and S100B more than 2-fold compared to those of freshly enriched hDSPCs by collagen type IV, suggesting that a monolayer culture cannot retain the stemness of hDSPCs (Fig. 1). Interestingly, we found that the formation of spheroid aggregations of hDSPCs in suspension culture significantly increased the expression levels of SOX2 and S100B mRNA compared to those of collagen type IV-enriched hDSPCs (Figs. 1A and 1C), indicating that 3D culture condition is appropriate for improving the stemness of hDSPCs.

Effects of glucose concentration on the stemness of hDSPCs

Previous studies reported that human bone marrow derived mesenchymal stem cells (hBM-MSCs), another type of mesenchymal stem cells, are grown and maintained in DMEM containing low glucose (1 g/L) (Chen et al., 2007; Shin et al, 2009). Thus, we performed real-time quantitative RT-PCR to determine if glucose concentration has effects on the gene expression levels of specific markers for hDSPCs under the 3D culture condition. DMEM supplemented with a low glucose (1 g/L) had no effect on the expression level of SOX2 (Fig. 2A). On the contrary, DMEM containing a high concentration of glucose (4.5 g/L) significantly increased the level of SOX2 gene transcript compared to the value of collagen type IV-enriched hDSPCs (Fig. 2A). The expression level of NANOG mRNA was relatively well retained by high glucose treatment, whereas it was significantly decreased under low glucose condition (Fig. 2B). In contrast, we found that the mRNA level of S100B was enhanced compared to that of collagen type IV-enriched hDSPCs, regardless of glucose concentration (Fig. 2C).

Effects of oxygen in maintaining the stemness of hDSPCs

Recent reports suggested that hypoxia contributes to maintain the stemness of MSCs (Grayson et al., 2007; Ren et al., 2006). Thus, we tested the effects of oxygen level on dermal progenitor markers and found that there were no significant differences between normoxic (20% of O_2) and hypoxic (1% or 5% of O_2) conditions in the mRNA expression levels of SOX2, NANOG and S100B (Supplementary Figs. 1A-1C). These results indicate that the stemness of hDSPCs did not depend on oxygen levels for cells grown *in vitro*. Initial *in vitro* experiments *confirmed* the stimulatory effect of *hypoxia* on *VEGF expression* (Minchenko et al., 1994). Thus, we also demonstrated that VEGF

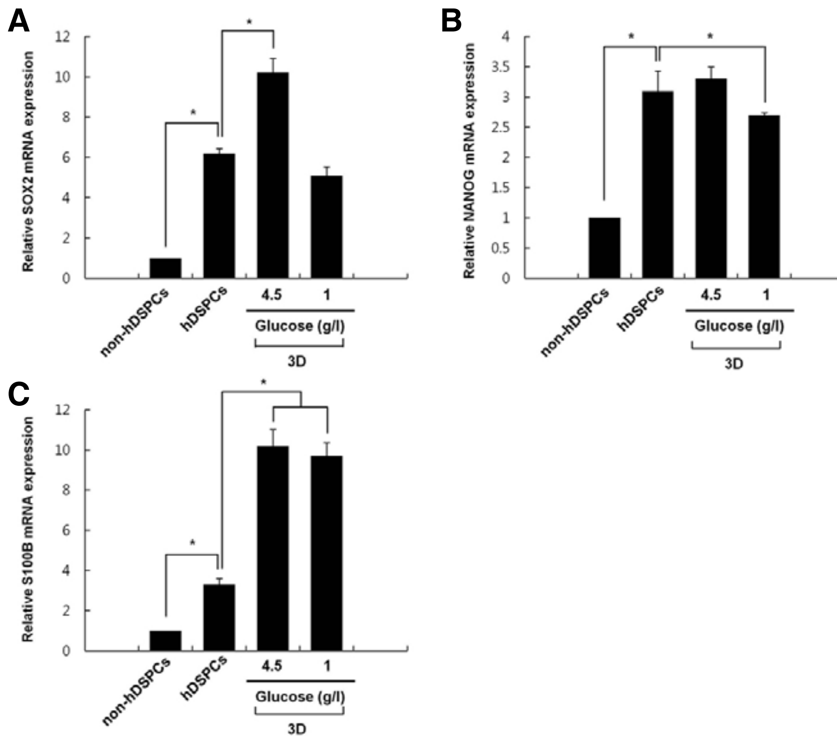

Fig. 2. Effects of glucose concentration on hDSPCs. Collagen type IV-enriched hDSPCs were cultured in two different glucose (1 g/L or 4.5 g/L) concentrations for 48 h. Total RNA was extracted, and real-time quantitative RT-PCR was performed to measure SOX2 (A), NANOG (B) and S100B (C). Values represent the mean expression ± S.D. of three independent experiments. *p < 0.01.

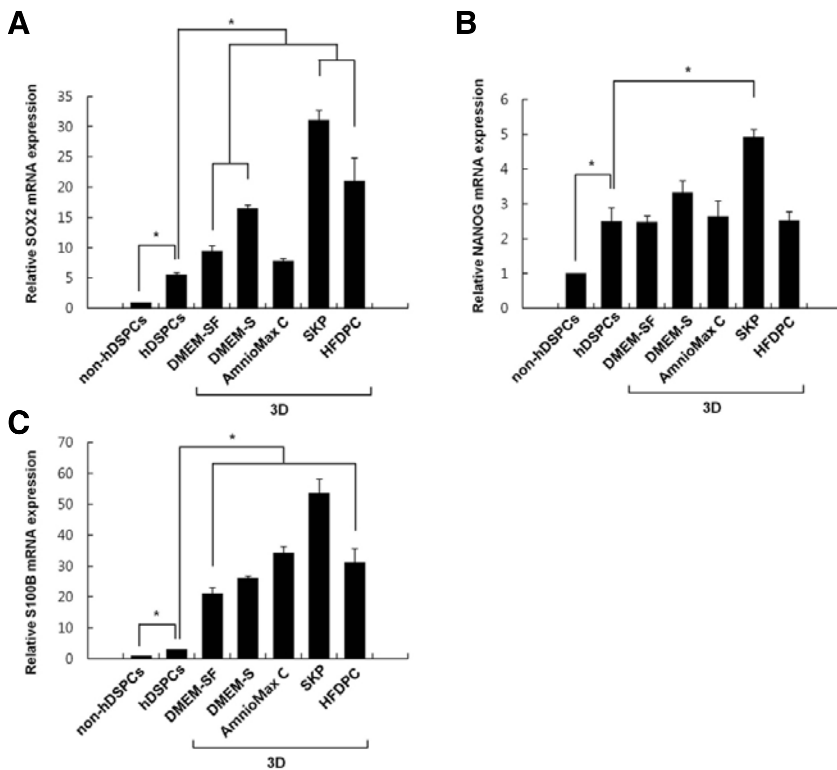

Fig. 3. Effects of various culture media on hDSPCs. Collagen type IV-enriched hDSPCs were cultured in five different media for 48 h. Total RNA was extracted, and real-time quantitative RT-PCR was performed to measure SOX2 (A), NANOG (B) and S100B (C). Values represent the mean expression ± S.D. of three independent experiments. *p < 0.01.

mRNA expression was increased in our hypoxic condition (Supplementary Fig. 1D).

Optimal medium formulation for maintaining the stemness of hDSPCs

We cultured hDSPCs in four commonly available culture media and compared them with serum-free DMEM for their effects on

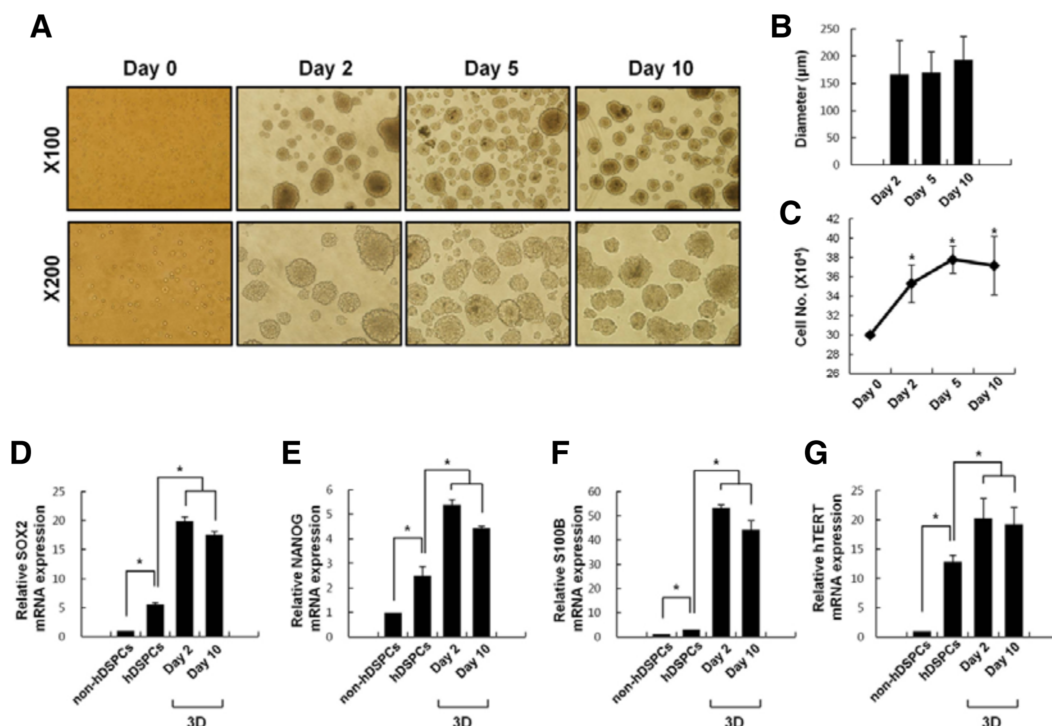

Fig. 4. Characterization of hDSPCs under a novel *in vitro* culture condition. Collagen type IV-enriched hDSPCs were cultured in SKP medium supplemented with high-glucose DMEM in a normoxic 3D culture condition during 10 days. Microscopic images of hDSPCs were shown at 0, 2, 5, 10 days after suspension culture (A). The diameter of hDSPCs obtained at 2, 5, 10 days was measured (B). The cell number of hDSPCs obtained at 0, 2, 5, 10 days was counted (C). Total RNA was extracted, and real-time quantitative RT-PCR was performed for SOX2 (D), NANOG (E), S100B (F), and hTERT (G). Values represent the mean expression ± S.D. of three independent experiments. *p < 0.01.

the stemness of hDSPCs. The four media evaluated were as follows: (i) DMEM containing 10% FBS; (ii) AmnioMAX C-100, which has been reported to maintain dermal papilla gene expression (Driskell et al., 2012; Rendl et al., 2005). (iii) skin-derived precursor (SKP) medium containing fibroblast growth factor 2 (FGF2) and epidermal growth factor (EGF) (Toma et al., 2001; 2005) and (iv) Hair follicle dermal papilla cell (HFDPC) growth medium supplemented with FGF2 and insulin (Reiter et al., 2008). Real-time quantitative RT-PCR was used to measure the mRNA levels of SOX2, NANOG, and S100B. We found that these dermal progenitor markers were significantly increased when cells were cultured in SKP medium compared to the other media (Fig. 3).

Novel *in vitro* culture condition enhanced expression levels of the dermal stem cell markers of hDSPCs

Taken together, these results suggested that suspension culture of hDSPCs with SKP medium containing high-glucose DMEM under normoxic conditions is associated with the expression of hDSPC-specific markers. To evaluate the culture condition for long-term maintenance of the stemness, hDSPCs were cultured for 10 days. The hDSPCs showed typical morphological changes, and started to aggregate themselves, and formed into sphere clearly by the 2 day of culture (Fig. 4A). We observed that the size of spheres was maintained similarly during 10 days (Fig. 4B). Interestingly, we found that the number of cells was gradually increased until day 5 compared to day 0, indicating that novel *in vitro* condition increased the proliferative property of hDSPCs (Fig. 4C). Some of the stemness markers were assessed by real-time quantitative RT-PCR. The mRNA expression levels of SOX2, NANOG and S100B at day 10 were relatively similar to the levels expressed at day 2 (Figs. 4D-4F). We further investigated the expression of human telomerase reverse transcriptase (hTERT), which plays an important role in maintaining unlimited cell proliferation. Interestingly, we found that hTERT mRNA expression increased significantly at day 2 and 10 in our optimized culture condition compared to that of collagen type IV-enriched hDSPCs (Fig. 4G).

A novel *in vitro* culture condition improved multipotency of hDSPCs

To examine whether multipotent property of hDSPCs is enhanced under this novel *in vitro* culture condition, hDSPCs obtained after 3D sphere formation (called as 3D-hDSPCs) were grown under specific culture conditions for differentiation of adipogenic and osteogenic lineages. Adipogenic differentiation was analyzed by measurement of Oil Red O staining after 10 days. The contents of Oil Red O demonstrated that 3D-hDSPCs could differentiate into mature adipocytes more efficiently than collagen type IV-enriched hDSPCs (Figs. 5A and 5B). The mRNA expression levels of PPARγ, adiponectin, leptin, and FABP4, which are expressed in mature adipocytes, were highly expressed in 3D-hDSPCs (Fig. 5C).

To verify osteogenic differentiation, ALP staining was performed. After being cultured in osteogenic medium for 2 weeks, 3D-hDSPCs could differentiate into mature osteoblasts more efficiently than collagen type IV-enriched hDSPCs (Fig. 5D). As shown in Fig. 5E, osteoglycin and osteocalcin mRNA expres-

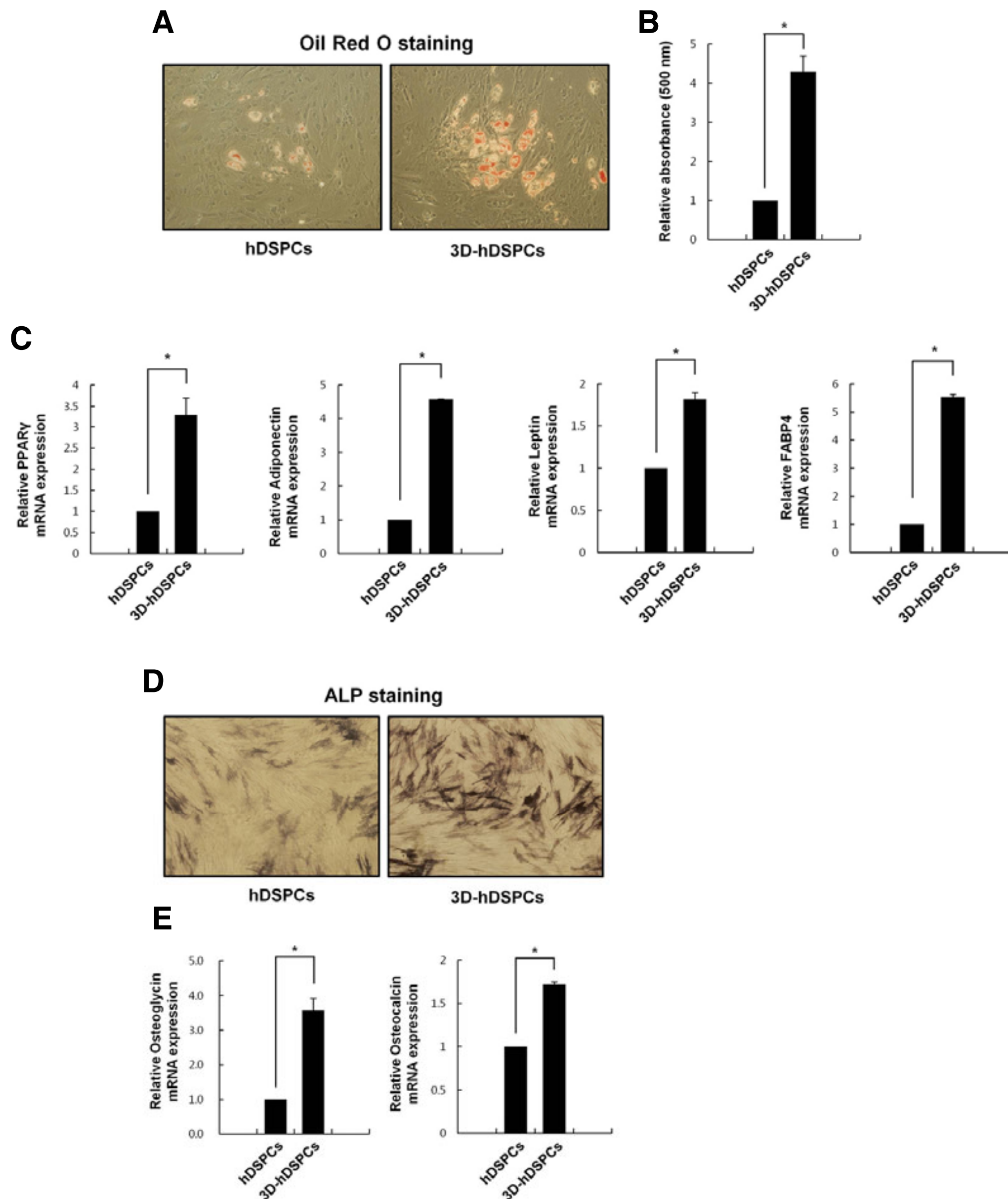

Fig. 5. Multipotency of hDSPCs obtained after 3D sphere formation under a novel *in vitro* culture condition. Collagen type IV-enriched hDSPCs were cultured in SKP medium containing high-glucose DMEM in a normoxic 3D culture condition for 2 days (called as 3D-hDSPCs). Collagen type IV-enriched hDSPCs and 3D-hDSPCs were cultured in adipogenic-inducing medium for 10 days. Oil Red O staining was positive in each sample (× 200) (A). Oil Red O was extracted from each sample and was measured at 500 nm (B). Relative expressions of PPARγ, adiponectin, leptin, and FABP4 were determined for each sample (C). Collagen type IV-enriched hDSPCs, and 3D-hDSPCs were cultured in osteogenic-inducing medium for 2 weeks. Alkaline phosphatase staining was positive in each sample (× 200) (D). Relative expressions of osteoglycin and osteocalcin were determined for each sample (E). The data repre-sent the mean ± S.D. of three independent experiments. $^{*}p < 0.01$.

sion levels were significantly increased in the 3D-hDSPCs after induction. Taken together, these results suggest that this novel culture condition contribute to improve the multipotency of hDSPCs.

DISCUSSION

Many attempts have been made to develop specific culture procedures for maintaining the stemness of adult stem cells including mesenchymal stem cells, such that the cells could be used for cell therapy (Jiang et al., 2002a; Pereira et al., 1995; Pittenger et al., 1999).

During *in vitro* culture, hDSPCs, which exhibited stem cell-like characteristics in our previous study, gradually lose the expression of transcription factors associated with multipotency and self-renewal, such as SOX2 and NANOG for maintaining the characteristics of stem cells. Several previous reports suggested that the quality of the stem cells strongly depend on the

culture method (Driskell et al., 2012; Grayson et al., 2007; Kuroda et al., 2010; Minchenko et al., 1994; Reiter et al., 2008; Ren et al., 2006; Rendl et al., 2005). Thus, we investigated whether hDSPCs retain their stemness in various culture conditions despite long-term culture.

In general, the property of MSCs depends upon the types of coating materials under *in vitro* culture conditions (Chen et al., 2007; Kim et al., 2013). In our study, we found that there is no significant difference between collagen types, which are representative extracellular matrices present in skin dermis. On the contrary, the stemness of hDSPCs seemed to be enhanced in 3D culture condition compared with other 2D culture conditions. The results indicated that the expression levels of several dermal progenitor gene markers, SOX2, NANOG, and S100B, were upregulated in 3D culture condition compared to those of freshly enriched hDSPCs by collagen type IV (Fig. 1). We confirmed that this phenomenon also occurred when hDSPCs were grown in another 3D culture system, using poly-HEMA plates (Supplementary Fig. 2). These results suggest that sphere formation of hDSPCs contributes to preserve the stemness property and the regenerative potential, which would be useful for therapeutic use.

According to previous results, oxygen is considered an important biochemical signaling molecule and a major regulator during development (Ezashi et al., 2005). It regulates various cellular events critical to the *in vitro* growth of cells. In the previous studies, hypoxia enhances the stemness of bone marrow mesenchymal stem cells (Grayson et al., 2007; Ren et al., 2006). Thus, we examined whether O_2 concentration had effects on preserving the stemness of hDSPCs. Interestingly, we observed that there are no significant differences in the expression levels of specific progenitor markers among hDSPCs cultured in 1, 5, and 20% O_2 concentration (Supplementary Fig. 1). Given that these results differed from what was observed in mesenchymal stem cells, these results suggest that the particular response to *in vitro* hypoxia is cell type-dependent despite mesenchymal stem cells in the same category.

Various formulations of culture medium have been developed to retain the stemness of dermal stem cells including dermal papilla cells. Toma *et al* demonstrated that small punch biopsies of human scalp proliferate in response to SKP medium supplemented with FGF2 and EGF (Toma et al., 2005). Dermal papilla cells derived from hair follicle show higher proliferative property in AmnioMAX C-100 or HFDPC growth medium compared to 10% FBS-DMEM (Driskell et al., 2012; Reiter et al., 2008). In our study, we demonstrated that SKP medium exhibited higher expression level of dermal progenitor genes compared to other culture media, indicating that SKP medium is appropriate for preserving the stemness of hDSPCs (Fig. 3).

Many studies reported that telomere shortening during cell division is mainly due to a decrease in telomerase activity (Bodnar et al., 1998; Jaskelioff et al., 2011; Wyllie et al., 2000). That is, it is expected that cells with extensive telomere shortening have a limited ability to proliferate. Thus, telomere length is also well known to serve as a marker of aging, The human telomerase reverse transcriptase (hTERT) is used as another index for diagnosing telomere shortening, as hTERT expression inhibits senescence and supports unlimited proliferative property. Thus, to characterize hDSPCs for the ability to self-renew, we examined whether hTERT expression was well-maintained in hDSPCs grown in our optimized culture condition, SKP medium supplemented with high-glucose DMEM in a normoxic 3D culture. We demonstrated that hTERT mRNA expression was significantly increased in hDSPCs compared to that of freshly enriched hDSPCs by collagen type IV (Fig. 4G).

We previously reported that hDSPCs has the multipotency to differentiate into mesodermal lineages (Shim et al., 2012; 2013). Therefore, we demonstrated that differentiation rate of adipocyte and osteoblast were significantly accelerated in 3D-hDSPCs (Fig. 5), suggesting that this novel *in vitro* culture condition enhance the multipotency of hDSPCs.

In conclusion, we suggest that this novel *in vitro* condition provides a method of safely preserving the stemness of hDSPCs, which is necessary in utilizing hDSPCs for cell therapy, such as wound healing, hair follicle regeneration, atopic dermatitis treatment, and psoriasis treatment.

Note: Supplementary information is available on the Molecules and Cells website (www.molcells.org).

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