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REGULAR ARTICLE

In vitro expansion of human adipose-derived stem cells in a spinner culture system using human extracellular matrix powders

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Abstract Stem cell therapy requires large numbers of stem cells to replace damaged tissues, but only limited numbers of stem cells can be harvested from a single patient. To obtain large quantities of stem cells with differentiation potential, we explored a spinner culture system using human extracellular matrix (hECM) powders. The hECM was extracted from adipose tissue and fabricated into powders. Human adipose-derived stem cells (hASCs) were isolated, seeded on hECM powders, and cultivated in a spinner flask. The 3-D culture system, using hECM powders, was highly effective for promoting cell proliferation. The number of hASCs in the 3-D culture system significantly increased for 10 days, resulting in an approximately 10-fold expansion, whereas a traditional 2-D culture system showed just a 2.8-fold expansion. Surface markers, transcriptional factors, and differentiation potential of hASCs were assayed to identify the characteristics of proliferated cells in 3-D culture system. The hASCs expressed the pluripotency markers, *Oct-4* and *Sox-2* during 3-D culture and retained their capacity to differentiate into adipogenic, osteogenic, and chondrogenic lineages. These findings demonstrate that the 3-D culture systems using hECM powders provide an efficient in vitro environment for stem cell proliferation, and could act as stem cell delivery carriers for autologous tissue engineering and cell therapy.

Keywords Adipose-derived stem cell · hECM powders · 3-D culture system · Large expansion · Autologous tissue engineering · Human

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Introduction

Adult mesenchymal stem cells (MSCs) have been isolated from mesodermal tissues including bone marrow, umbilical cord blood, muscle, brain, and adipose tissue. These cells have multilineage potential, exhibiting adipogenic, osteogenic, chondrogenic, myogenic, and neurogenic differentiation (Jiang et al. 2002). Recently, interest in adipose tissue has grown rapidly because adipose-derived stem cells (ASCs) with multi-differentiation potential can be easily harvested (Zuk et al. 2002). Indeed, adipose tissue is practical, abundant, expendable, and can be safely obtained via

minimally invasive operations using liposuction techniques. Bone marrow (BM) is a major current source for multipotent MSCs, but harvesting BM-MSCs is more invasive. Therefore, ASCs are a promising stem cell source for autologous cell therapy and stem cell-based biotechnology.

In many cases, cell culture techniques may be required to increase the number of stem cells available because stem cell therapy requires a large amount of stem cells to replace damaged tissues. Stem cell expansion depends not only on natural factors, like the condition of the donor, but also on technical factors, such as the isolation procedure and in vitro culture conditions (Bruder et al. 1997). Adult stem cell expansion typically includes multiple steps in two-dimensional (2-D) conditions. The traditional 2-D culture model suffers from cell supply and expansion limitations resulting from various factors, such as the time requirements, costs of maintenance, and culture environment that is very different from in vivo contexts. In addition, cells are known to lose their phenotypes and change their biochemical characteristics in long-term 2-D culture (Sart et al. 2009).

Microenvironmental signaling of the native tissue, including cell–cell, cell–matrix, and biological signal molecule interactions, regulates expansion and differentiation of many cell types (Discher et al. 2009). Researchers have tried to develop three-dimensional (3-D) culture systems mimicking in vivo tissues with regard to cell shape and cellular environment for tissue engineering, scale-up production of cells and their products, and clinical stem cell trials (Godara et al. 2008; King and Miller 2007; Zangi et al. 2006). Spinner culture systems have significant advantages. They provide a more homogeneous culture environment, control of pH, oxygen concentration and flow shear stress, and scalable culture environments (Fok and Zandstra 2005; Hu and Aunins 1997; Sart et al. 2010). Moreover, spinner culture systems have been used to expand MSCs with engineered 3-D scaffolds such as microcarriers, providing a physical support matrix and increasing cell–cell and cell–substrate interactions (Eibes et al. 2010; Frauenschuh et al. 2007). The most common microcarriers have been prepared from alginate (Murua et al. 2008), dextran (Phillips et al. 2008), collagen (Wu et al. 2007a), gelatin (Akasha et al. 2008), fibrin (Abranches et al. 2007), polystyrene (Lee et al. 1992), polyester (Xu and Reid 2001), and hybrid polymers (Wu et al. 2007b). Microcarriers prepared from extracellular matrix (ECM) components have received increasing attention due to their excellent biological properties, such as promotion or inhibition of cell proliferation and migration, triggering of matrix remodeling, and promotion of enhanced tissue organization and biocompatible properties, including low antigenicity, biodegradability, and non-toxicity (Badylak et al. 2009; Philp et al. 2005).

Recently, we have developed new ECM scaffolds composed of human adipose tissue extracts (Choi et al. 2009, 2010). Adipose tissue is abundant in human body and contains not only cellular components, such as ASCs, fibroblast, and adipocytes but also various ECM components, such as collagen, reticular fibers, elastin fibers, nerve fibers, vascular stroma, and lymph nodes (Ahima and Flier 2000). ECM components can be extracted from human adipose tissue by a simple mechanical process and fabricated into various shapes such as sponges, sheets, cubes, tubes, and powders. In this paper, we describe the expansion of hASCs using hECM powders derived from adipose tissue. We have developed and tested a spinner culture system using hECM powders to provide an efficient environment for 3-D stem cell growth.

Materials and methods

Fabrication of human ECM powders from adipose tissue

Human ECM powders were prepared from adipose tissue as described in our previous study (Choi et al. 2009). Briefly, human adipose tissue was obtained from healthy donors between 20 and 40 years of age who had undergone liposuction with a single combined machine (Lipokit; Medikan, Seoul, Korea) at the Kangnam Plastic Surgery Clinic (Seoul, Korea). The adipose tissue obtained by liposuction (20 mL) was washed three times with distilled water to remove blood components. Distilled water (10 mL) was added to the adipose tissue, and the tissue/water mixture was homogenized at 12,000 rpm for 5 min using a homogenizer (T 18 basic ULTRA-TURAX; IKA®-Werke, Staufen, Germany). The tissue suspension was centrifuged at 1,800*g* for 5 min and the upper layer containing oil components was discarded. This process was repeated several times. The viscous tissue suspension was washed three times with distilled water, frozen at −70°C, freeze-dried, and crushed using a manual mill.

hASC isolation and maintenance

According to a modified procedure (Bunnell et al. 2008), the adipose tissue was washed with phosphate-buffered saline (PBS) containing 5% penicillin/streptomycin (P/S) (Gibco-BRL Life Technologies, Carlsbad, CA, USA). After removing red blood cells, the adipose tissue was digested in PBS supplemented with 0.01% (w/v) collagenase type II (Gibco-BRL) for 1 h at 37°C. The digested tissue was filtered through a 100-μm mesh to remove aggregated tissue and debris. The filtered suspension was centrifuged at 200*g* for 7 min and the resulting stromal vascular fraction (SVF) pellet was washed several times in PBS. SVF cells were incubated in Dulbecco's Modified Eagle's Medium (DMEM; Gibco-BRL) supplemented with 10% fetal bovine

serum (FBS; Gibco-BRL) and 1% P/S at 37°C under 5% CO_2. The cells were maintained until passage 2.

3-D culture

hECM powders were sterilized by ethylene oxide (EO) gas, washed in PBS and swelled with serum free DMEM. For 3-D culture, the hECM powders (500 mg) were pre-incubated for 30 min with DMEM in a spinner flask (250 mL) containing a magnetic stirring bar (80 mm^2; Corning, Somerset, NY, USA). A total of 2×10^6 hASCs were seeded onto the hECM powders in maintenance medium composed of DMEM, 10% FBS, and 1% P/S. After a 12-h attachment period without stirring, 50 mL of maintenance medium was added to a spinner flask on a magnetic stirring plate (Bellco Glass, Vineland, NJ, USA) at 80 rpm in an incubator. The culture medium was half-removed and replaced by fresh medium, every 2 days. The cultures were maintained at 37°C under 5% CO_2 for 10 days.

Cell adhesion and proliferation

The cell number attached on hECM powders was evaluated using an automatic cell counter (NucleoCounter™; Chemometec, Allerød, Denmark), by staining with propidium iodide (PI), which stains cell nuclei. One-mL samples were taken out in quintuplicate from each spinner flask, mixed with equal volumes of cell lysis buffer, and then stabilizing buffer. An aliquot of the mixture (100 μL) was loaded into a cassette of the automatic cell counter, which is an integrated fluorescence microscope designed to detect signals from PI bound to DNA.

Cell viability assay

The cell viability analysis was performed using a commercially available Live/Dead® Viability/Cytotoxicity kit (Molecular Probes, Eugene, OR, USA). The cell-seeded hECM powders were transferred to a new plate, washed in PBS, and stained for 30 min with 100 μL of the combined Live/Dead® reagents at 37°C. After staining, the cell-seeded hECM powders were observed using a fluorescence microscope (IX81; Olympus, Tokyo, Japan). Green fluorescence caused by a calcein reaction with intracellular esterase indicated live cells, whereas red fluorescence caused by ethidium homodimers that bound to nucleic acids indicated dead cells.

Scanning electron microscopy

The morphology of cells attached to hECM powders was observed using a scanning electron microscope (SEM; Hitachi S-4800 FE-SEM, Tokyo, Japan). Samples obtained from a spinner flask were washed with PBS, fixed in 2.5% glutaraldehyde, dehydrated through a graded ethanol series, frozen at −70°C, and freeze-dried. Samples were fixed to metal stubs and coated with platinum by a sputter at an accelerating voltage of 15 kV.

Flow cytometry analysis

Freshly isolated SVF and 2-D cultured cells were harvested using 0.05% trypsin-EDTA (w/v) (PAA Laboratories, Wagram, Pasching, Austria) and 3-D cultured cells were detached from hECM powders using collagenase (type II; Gibco-BRL) digestion. 0.01% Collagenase type II was added to each 3-D culture sample and incubated in a 37°C shaking water bath for 30 min. The digested samples were then centrifuged at 200*g* for 5 min. The supernatant was removed and the pellet was washed in PBS. Cell samples were washed in cold PBS, re-suspended at a concentration of 1×10^5 cells in a final volume of 500 μL, and incubated at 4°C for 1 h with 10 μL of each antibody with fluorochrome-conjugated antibodies against the detected surface markers or an isotype control. Antibodies were purchased from BioLegend (San Diego, CA, USA): APC anti-human CD29, FITC anti-human CD34, and FITC anti-mouse/human CD44. Cells were washed, re-suspended in PBS, and analyzed with a FACSCalibur™ flow cytometer (BD Biosciences, San Jose, CA, USA).

Multilineage differentiation of hASCs

Multilineage differentiation of hASCs was induced with different media in 2-D culture dishes (Katz et al. 2005; Yañez et al. 2006). All supplements were purchased from Sigma-Aldrich (St. Louis, MO, USA). hASCs were harvested from hECM powders, re-seeded on culture dishes, and cultured until confluent. For adipogenic differentiation, hASCs were maintained in adipogenic medium (AM) composed of DMEM supplemented with 10% FBS, 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), 1 μM dexamethasone, 10 μM insulin, 200 μM indomethacin, and 1% antibiotic. After 3 days, the medium was replaced by adipogenic maintenance medium without IBMX. For osteogenic differentiation, hASCs in a six-well plate were incubated in osteogenic medium (OM), DMEM supplemented with 10% FBS, 0.1 μM dexamethasone, 50 μM ascorbate-2-phosphate, 10 mM β-glylcerophosphate, and 1% antibiotic. To induce chondrogenic differentiation, hASCs were cultured in a six-well plate at a high density (2×10^6 cells/well). The chondrogenic medium contained DMEM supplemented with 10% FBS, 6.25 μg/mL insulin, 10 ng/mL TGF-β_1, 50 nM ascorbate-2-phosphate, and 1% antibiotic. Cells were maintained in differentiation media for 2 weeks, with the media replaced every 2 days.

Reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was isolated from cells using Trizol reagents (Invitrogen, Karlsruhe, Germany). The complementary DNA (cDNA) was synthesized from 1 μg total RNA using Molony-Murine Leukemia Virus Reverse Transcriptase (M-MLV RT; Invitrogen). The cDNA was used as a template for PCR analysis using primers. PCR amplification was performed in a GeneAmp® PCR 9700 (Applied Biosystems, Foster City, CA, USA) instrument under the following reaction profiles: initial denaturation at 95°C for 5 min and 35 cycles of denaturation at 95°C for 40 s, annealing at 52–60°C for 1 min, and extension at 72°C for 10 min. As an internal control, GAPDH expression was assessed using primers designed for the housekeeping gene. PCR products were electrophoresed in 2% agarose gels and analyzed using a gel imaging system (Gel Logic 200; Kodak, Rochester, NY, USA) (Table 1).

Histological analysis

All supplements were purchased from Sigma-Aldrich. Cells were fixed in 2.5% paraformaldehyde, dehydrated in a graded ethanol series and washed with PBS. To identify adipogenesis, samples were incubated in cold acetone for 10 min and washed with 30% isopropanol. The lipid vacuoles were then stained by oil red O for 15 min and destained in 60% isopropanol. To observe calcified extracellular matrix deposits from osteogenesis, von Kossa staining was performed. Samples were stained with 5% silver nitrate solution under ultra-violet light for 45 min and treated with 3% sodium thiosulphate. Chondrogenic differentiation was confirmed by Alcian blue staining for sulfate proteoglycans. Samples were stained with Alcian blue solution for 30 min and destained in 100% isopropanol. All samples were followed by counter staining with nuclear fast red or hematoxylin.

Statistical analysis

Experimental data were expressed as means ± standard deviation (SD). Student's two tailed *t* test with SPSS 17.0 statistical software (SPSS, Chicago, IL, USA) was used for comparison, and statistical significance was accepted at $p<0.01$.

Results

3-D cell culture systems using human ECM powders

We attempted 3-D hASC culture using a powdered scaffold derived from human adipose tissue. The overall strategy is schematically represented in Fig. 1. Human adipose tissue was obtained by liposuction. hASCs were isolated from a

Table 1 Primer sequences for RT-PCR analysis

Gene	Forward and reverse primer sequences	Annealing temperature	Product size (base pair)	Gene bank accession no.
Housekeeping gene				
GAPDH	5′- GGG CTG CTT TTA ACT CTG GT-3′ 5′- GCA GGT TTT TCT AGA CGG-3′	56°C	702 bp	NM002046
Pluripotency-related genes				
Oct-4	5′- CGC ACC ACT GGC ATT GTC AT-3′ 5′- TTC TCC TTG ATG TCG CGC AC-3′	55°C	205 bp	NM002701
Sox-2	5′- GGC AGC TAC AGC ATG ATG CAG GAC C-3′ 5′- CTG GTC ATG GAG TTG TAC TGC AGG-3′	58°C	131 bp	NM003106
Adipogenic genes				
PPARγ	5′-AGA CAA CAG ACA AAT CAC CAT-3′ 5′-CTT CAC AGC AAA CTC AAA CTT-3′	50°C	401 bp	NM015869
aP2	5′-TGC AGC TTC CTT CTC ACC TTG A-3′ 5′-TCC TGG CCC AGT ATG AAG GAA ATC-3′	55°C	256 bp	NM001442
Osteogenic genes				
ALP	5′-TGG AGC TTC AGA AGC TCA ACA CCA-3′ 5′-ATC TCG TTG TCT GAG TAC CAG TCC-3′	60°C	454 bp	NM000478
Chondrogenic genes				
AGN	5′-TGA GGA GGG CTG GAA CAA GTA CC-3′ 5′-GGA GGT GGT AAT TGC AGG GAA CA-3′	60°C	350 bp	NM013227

GAPDH glyceraldehyde 3-phosphate dehydrogenase, *Oct-4* Octamer-4, *Sox-2* SRY (sex determining region Y)-box 2, *PPARγ* peroxisome proliferator-activated receptor gamma, *aP2* adipocyte fatty acid-binding protein, *ALP* alkaline phosphatase, *AGN* aggrecan

Fig. 1 Schematic representation of a 3-D culture system using hASCs and hECM powders derived from adipose tissue

portion of adipose tissue by an enzymatic digestion method using collagenase, and then expanded until passage 2. hECM powders were fabricated from the remaining portion of adipose tissue by homogenization, centrifugation, and freeze-drying. The isolated hASCs were seeded on hECM powders and incubated in a spinner flask. The 3-D culture system is expected to lead to a new autologous tissue engineering strategy, in which both biomaterials and stem cells are extracted from the adipose tissue of the same patient.

hASC proliferation on hECM powders

hASCs can be easily obtained from human adipose tissue, but various factors such as donor age, local origin of the adipose tissue, type of surgical procedure, culture conditions, plating density, and media formulations are known to influence both proliferation rate and differentiation capacity of hASCs (Schäffler and Büchler 2007). Thus, before seeding on hECM powders, hASCs were expanded in a general culture dish up to passage 2. Then, the cells were mixed with hECM powders and incubated. The percentage of cells attached to hECM powders in the 3-D system was slightly lower than those observed in a 2-D culture dish (Fig. 2a). As shown in Fig. 2b, the expansion curves of the two culture methods differed significantly. The cell number after 10 days in the 3-D culture system showed an approximately 10-fold expansion, whereas a traditional 2-D culture system showed just a 2.8-fold expansion.

The hASCs cultured with hECM powders showed good viability and distribution, as confirmed by a live/dead assay (Fig. 3a, b). Green fluorescence by calcein reaction with an intracellular esterase indicates live cells, whereas red fluorescence from ethidium homodimers that bind to nucleic acids indicates dead cells. During the initial culture period, hASCs attached to hECM powders (Fig. 3a). Cells proliferated on the surface of hECM powders and grew into spindle-shaped cells (Fig. 3b). Especially, hASCs formed a network structure due to interaction with neighboring cells and with hECM powders on day 10 (Fig. 3c, d).

Identification of hASC pluripotency

The hASC pluripotency changes with adherence and passage. Generally, hASCs express not only immunophenotype

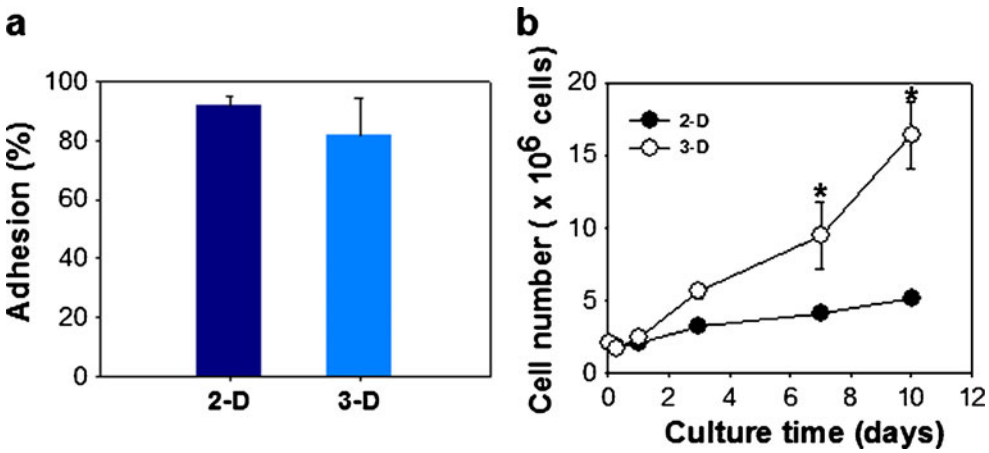

Fig. 2 **a** Cell attachment percentages 6 h post-seeding. **b** Proliferation kinetics for 10 days in 2-D and 3-D culture systems of hASCs. For 3-D culture, a suspension containing 2×10^6 cells was seeded on hECM powders (500 mg). As a control group (2-D culture), hASCs were seeded in a 100 mm culture dish. The number of cells was measured by an automatic cell counter. Each point represents the mean and standard deviation of $n=5$ independent replicates (*$P<0.01$)

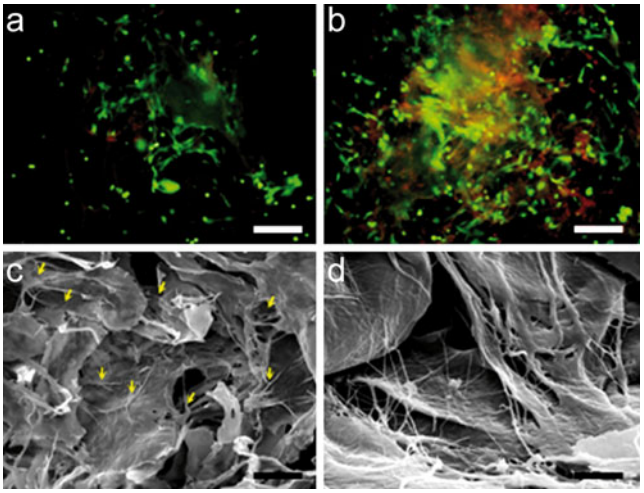

Fig. 3 **a**, **b** Fluorescence micrographs and **c**, **d** SEM images of hASCs expanded on hECM powders on day 1 (**a**, **c**) and day 10 (**b**, **d**). Cells were stained by calcein-AM (*green* live) and ethidium homodimer (*red* dead). *Scale bars* 100 μm

markers such as adhesion molecules (CD29 and CD49e), receptor molecules (CD44), cadherins (CD144), surface enzymes (CD73), extracellular matrix proteins (CD90 and CD105), intercellular adhesion molecules (CD54), vascular adhesion molecules (CD106), complement regulatory proteins, and histocompatibility antigens (HLA-DR) (Katz et al. 2005; Yañez et al. 2006) but also transcription factors such as *Oct-4*, *Sox-2*, and *Nanog* (Zhu et al. 2008). Figures 4 and 5a show the representative flow cytometry histograms for cell surface markers and RT-PCR for transcription factor expression pattern, respectively. The hASC at passage 2 expressed undifferentiated MSC markers, CD29 and CD44. The hASCs expressed CD29 and CD44 during culture periods (10 days) in both 2-D and 3-D culture systems. The hematopoietic marker, CD34, was expressed slightly in the SVF cells (13%) and hardly detected in both 2-D and 3-D culture systems. The mRNA of transcription factors (*Oct-4* and *Sox-2*) of hASCs in the 3-D culture system were also clearly expressed until 10 days (Fig. 5a). The results imply that the immunophenotype and transcription factors expression patterns were similar in 2-D and 3-D culture systems and hASC retained their pluripotency after expansion in 3-D culture.

Multilineage differentiation potential of hASCs

The differentiation potential of hASCs cultured in the 3-D system was analyzed by RT-PCR (Fig. 5b). After 10 days in a spinner flask, hASCs were harvested from hECM powders, re-seeded on culture dishes, and then cultured with each

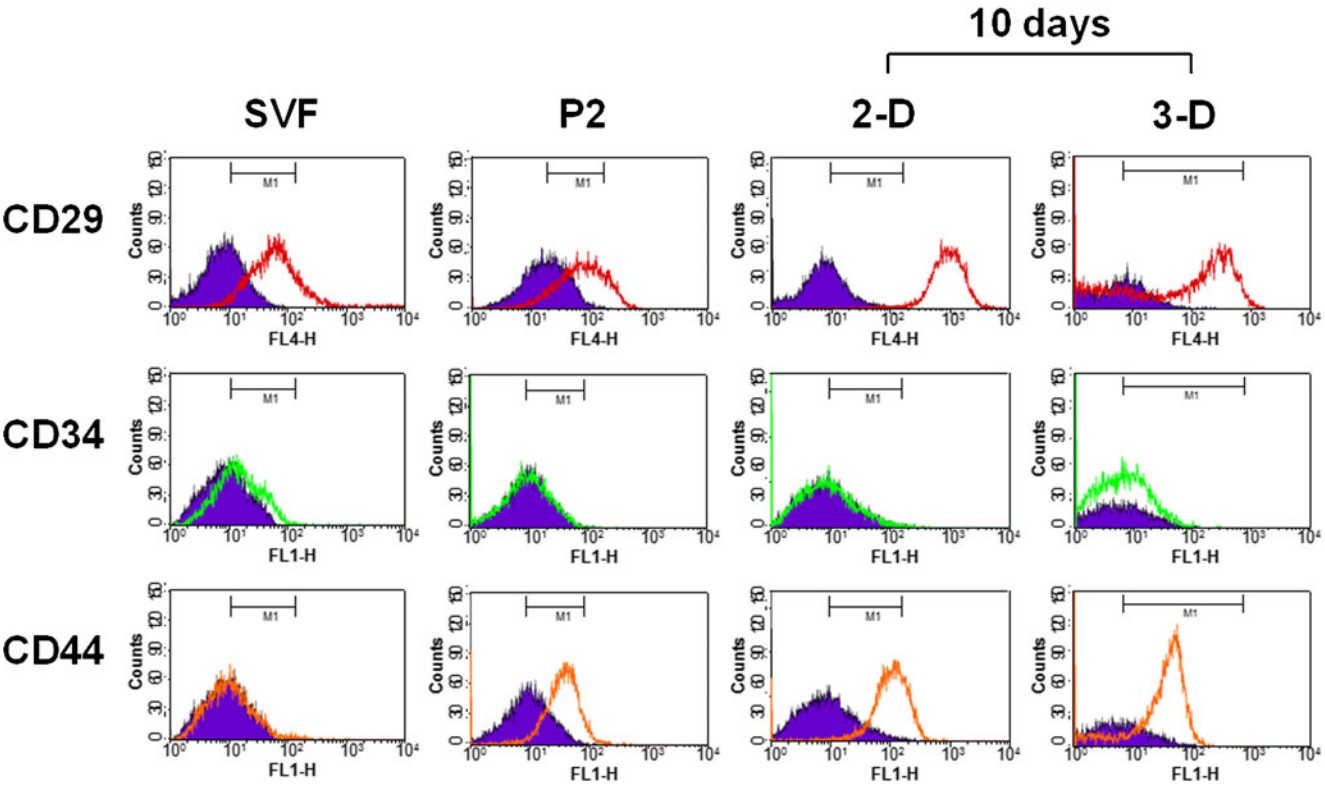

Fig. 4 Representative flow cytometry histograms for cell surface. The flow cytometry histograms for undifferentiated MSC markers (*CD29* and *CD44*) and a hematopoietic marker (*CD34*) were represented on freshly isolated SVF cells, hASCs at passage 2, hASCs in 2-D and 3-D culture for 10 days. Each *color line* indicates positive staining cells, whereas the *purple area* indicates the isotype-matched monoclonal antibody control

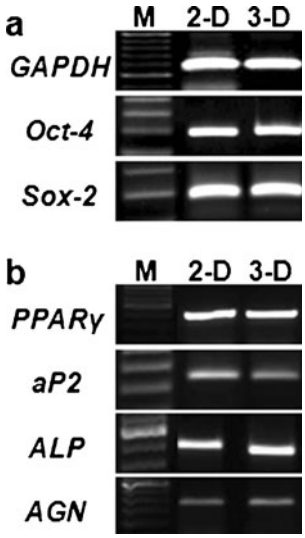

Fig. 5 RT-PCR analysis for multilineage differentiation of hASCs after expansion in 3-D culture. **a** RT-PCR analysis of gene expression for transcription factors on the 10th day in 3-D culture. **b** The multilineage differentiations of hASCs expanded in 3-D culture were induced with specific media containing adipogenic, osteogenic, and chondrogenic differentiation factors. Each expressed gene in RT-PCR was analyzed using human-origin primers designed for transcription factors, adipogenic, osteogenic, and chondrogenic specific genes. *GAPDH* was used for normalization

differentiation medium containing specific growth factors into adipogenic, osteogenic, and chondrogenic lineage. After treatment with each differentiation medium, hASCs expressed multiple genes with adipogenic, osteogenic, and chondrogenic lineage characteristics. Specific expression of *PPARγ* and *aP2* confirmed adipogenesis. Furthermore, expression of both osteogenic (*ALP*) and chondrogenic genes (*AGN*) were detected in hASCs expanded in the 3-D culture system.

Moreover, hASC multi-differentiation was evaluated by histological staining (Fig. 6). After treatment with adipogenic differentiation medium, hASCs accumulated lipid vacuoles, which stained positive by oil red O. Osteogenic potential was assessed by von Kossa staining, and deposition of mineralized bone matrix appeared. To assess chondrogenic potential cells were stained with Alcian blue, and cartilage proteoglycan deposition was observed.

Discussion

3-D culture techniques using scaffolds are rapidly evolving with the goal of simulating in vivo environments for tissue engineering and regenerative medicine. In this study, we investigated hASC response when cultured using ECM scaffolds derived from human adipose tissue in a spinner flask. A wide variety of biomaterials have been utilized to produce micro-scaffolds. The ideal biomaterials should provide a suitable environment for cell adhesion and proliferation to cells or implanted tissues (Hench and Polak 2002). The surface properties of biomaterials have a significant influence on cell–cell and cell–matrix interactions that mediate subsequent cell responses (Anselme and Bigerelle, 2006). Hence, the choice of biomaterials in 3-D culture is critical for stem cell adhesion, proliferation, and differentiation. In previous studies (Choi et al. 2009, 2010), we reported that ECM scaffolds derived from human adipose tissue contain various ECM components such as collagen, glycosaminoglycan, and elastin. The hECM powders in the present study have an extensive surface area and provide a strong but non-rigid substrate for stirred culture. A powdered form of scaffold provides favorable surfaces for efficient attachment and spreading of animal cells (Gilbert et al. 2005). The hASCs, which were also isolated from human adipose tissue, exhibited good adhesion, spreading, and proliferation in 3-D culture with

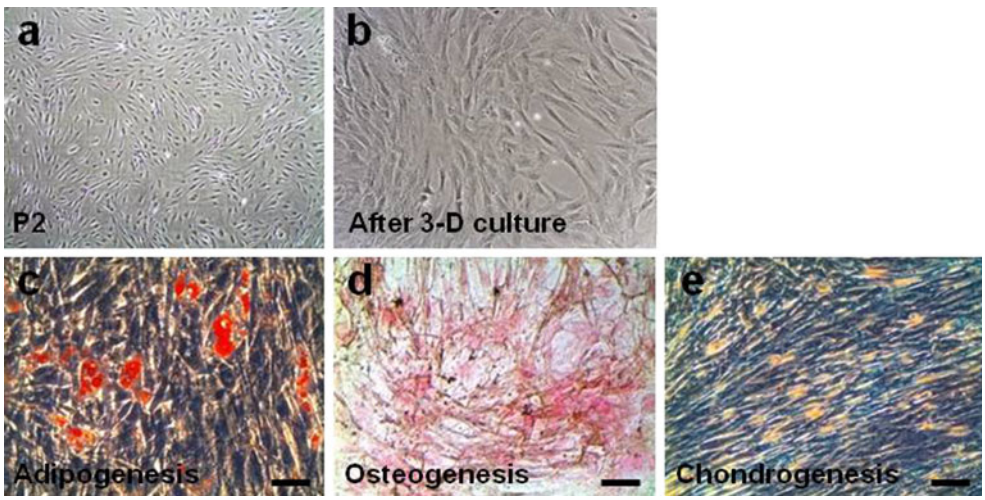

Fig. 6 **a**–**e** Histochemistry staining for multilineage differentiation of hASCs after expansion in 3-D culture. The multilineage differentiations of hASCs expanded in 3-D culture were induced with specific media containing adipogenic, osteogenic, and chondrogenic differentiation factors. The multilineage differentiations were confirmed as follows: **c** lipid vacuoles accumulated in cells stained by oil red O (*red*) for adipogenesis; **d** calcified extracellular matrix deposits (*black or dark brown*) stained by von Kossa for osteogenesis; **e** sulfate proteoglycans (*blue to bluish green*) stained by Alcian blue for chondrogenesis. *Scale bars* 100 μm

hECM powders (Figs. 2 and 3). While the cell adhesion in 3-D culture was slightly lower than in 2-D culture, the cell growth rate in 3-D culture was significantly faster than in 2-D culture. The cell growth is generally regulated not only by cell adhesion to the solid substrate but also by microenvironments such as growth factors, components of the scaffolds, ingredients of the culture medium, and culture condition (Chai and Leong 2007; Curran et al. 2005; Godara et al. 2008). Various cell–cell and cell–surface interactions and soluble factors have been investigated with the aim of developing in vivo-like environments for large expansion and differentiation of stem cells. Collagen, a major component of hECM powders and endogenous growth factors may promote the signal transduction between cells and the scaffold, which benefits the expansion of stem cells. In addition, the spinner culture system provides homogeneous and scalable culture environments, which can increase cell–cell and cell–hECM powders interactions.

Generally, hASCs with multipotent differentiation potential appear promising as a source of autologous stem cells. hASCs could be expanded in vitro up to 120 cell divisions. In order to obtain sufficient cells for therapeutic purposes, extensive in vitro expansion would be required (Zuk et al. 2001). As a natural component in the body, ECM may promote signal transduction between cells or cell–matrix, which influences adhesion, migration, proliferation, and differentiation (Badylak et al. 2009). Therefore, the surface features and chemical and biological compositions of hECM powders could support hASC adhesion and proliferation. The present study implies that in vitro mass stem cell proliferation could be achieved in a 3-D culture system using hECM powders to produce sufficient cells to engraft a patient.

Microenvironments play an important role in determining cell fates such as changing morphology, migration, proliferation, differentiation, and apoptosis. In particular, cell shape in culture is closely related to adult mesenchymal stem cell (MSC) differentiation potential (Estes et al. 2008; Zhao et al. 2009). For example, Sekiya et al. (2002) demonstrated that thin and spindle-shaped stem cells possess a high propensity for multilineage differentiation. In our system, hASCs cultured with hECM powders grew into spindle or stellate shapes (Fig. 3), whereas they appeared flat in a general 2-D culture system. Another important phenomenon is that the hASCs produced in the 3-D system retained stem cell characteristics up to 10 days, and underwent multiple differentiation into adipogenic, osteogenic, and chondrogenic lineages (Figs. 4, 5 and 6). After expansion in 3-D culture, hASCs were clearly positive for CD29 and CD44, and expressed *Oct-4* and *Sox-2* mRNA, which suggests that the cells retained their pluripotency. In addition, the RT-PCR analysis and histological staining confirmed the differentiation potential to adipogenic, osteogenic and chondrogenic lineages of hASCs. hASCs cultured in the 3-D system expressed specific differentiation markers such as oil droplet formation for adipogenic, mineralized bone matrix deposition for osteogenic, and cartilage proteoglycan deposition for chondrogenic markers. These data support that the 3-D culture system using hECM powders can produce a large amount of pluripotency stem cells for a relatively short period.

Conclusions

In this study, we have demonstrated that hECM powders provide suitable substrates for hASC adhesion and proliferation. The 3-D culture systems using hECM powders provide a highly favorable environment for large in vitro expansion of hASCs with pluripotency. The 3-D stem cell culture system described in this study is expected to provide a basis for producing stem cells in large numbers for cell therapy applications and improve the efficacy for autologous tissue engineering, in which both stem cells and tissue engineering scaffolds are derived from the same patient.

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