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Comparison of Endothelial Differentiation Capacities of Human and Rat Adipose-Derived Stem Cells

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Background: The authors compared the endothelial differentiation capacities of human and rat adipose-derived stem cells to determine whether human adipose-derived stem cells can be a source of endothelial cells clinically.

Methods: Human and rat adipose-derived stem cells were harvested and characterized with flow cytometry and trilineage differentiation. Cells from passages III through V were fed with endothelial cell differentiation medium for up to 3 weeks. Cells were harvested after 1, 2, and 3 weeks, and endothelial differentiation was evaluated with quantitative reverse-transcriptase polymerase chain reaction, flow cytometry, and angiogenic sprouting assays.

Results: Both human and rat adipose-derived stem cells were CD90⁺, CD44⁺, and CD31⁻ before differentiation. The cells were successfully differentiated into adipogenic, osteogenic, and chondrogenic lineages. Expression of endothelial cell-specific genes peaked at the second week of differentiation in both human and rat cells. The fold changes in expression of CD31, vascular endothelial growth factor receptor-1, nitric oxide synthase, and von Willebrand factor genes at week 2 were 0.4 ± 0.1 , 34.7 ± 0.3 , 2.03 ± 0.25 , and 12.5 ± 0.3 respectively, in human adipose-derived stem cells; and 1.5 ± 1.01 , 21.6 ± 1.7 , 17.9 ± 0.6 , and 11.2 ± 1.3 , respectively, in rat cells. The percentages of CD31⁺ cells were 0.2, 0.64, and 1.6 in human cell populations and 0.5, 5.91, and 11.5 in rat cell populations at weeks 1, 2, and 3, respectively. Rat adipose-derived stem cell-derived endothelial cells displayed enhanced sprouting capability compared with the human cells.

Conclusions: Human adipose-derived stem cells responded less strongly to EGM-2MV endothelial differentiation medium than did the rat cells. Still, the human cells have the potential to become a clinical source of endothelial cells with modifications in the differentiation conditions. (*Plast. Reconstr. Surg.* 138: 1231, 2016.)

Emerging interest in autologous stem cells and biotechnological advances have paved the way for the development of several tissue-engineered constructs for tissue regeneration.¹ However, the main roadblock preventing clinical translation of these regenerative methods is the lack of a vascular system within tissue-engineered constructs. In the initial period after transplantation, thin and small constructs

may successfully rely on passive diffusion for nutrition.^{2,3} However, larger and more complex constructs cannot survive by means of passive diffusion alone. Instead, they require a sophisticated vascular network for efficient and immediate

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nutrient delivery.^{2,4} New vessel ingrowth typically takes several days to establish a vascular network that can provide a sufficient blood supply to these thick constructs. In this time, the cells deepest in the tissue (i.e., >200 μm from the surface) and farthest from the blood supply may die, ultimately jeopardizing the survival of the entire construct.^{2,3}

An alternative strategy to overcome this problem is seeding the constructs with endothelial cells to accelerate the formation of a vascular network, thereby preventing the death of seeded cells and resorption of the construct.⁴ However, the use of autologous endothelial cells is hampered by the need to harvest a blood vessel from the patient and then isolate and culture enough endothelial cells to seed the construct.⁵ Fortunately, autologous mesenchymal stem cells have risen as an alternative source for different types of cells, including endothelial cells. Mesenchymal stem cells have the ability to differentiate into lineages of mesodermal tissues, such as skeletal muscle, bone, tendons, cartilage, and fat, under appropriate culturing conditions using specific hormones or growth factors, and can be isolated from several organs, such as fetal liver, umbilical cord blood, and bone marrow.^{5,6} To date, most attempts to

create vascularized tissue-engineered constructs with stem cells have used either endothelial progenitor cells harvested from blood, or stem cells harvested from bone marrow.⁷⁻⁹ Both of these cell populations have shown remarkable potential to modulate into endothelial-like cells; however, cell availability may be severely limited by advanced patient age and invasiveness of the harvesting methods.¹⁰ The use of adipose-derived stem cells as an autologous cell source circumvents these problems because these cells can readily be harvested from large subcutaneous deposits of adipose tissue with low donor-site morbidity.^{11,12} They can be induced toward endothelial cells *in vitro* in the presence of vascular endothelial growth factor (VEGF), and participate in vascular-like structure formation both in ischemic tissues and in Matrigel (Corning, Inc., Corning, N.Y.), as documented by numerous studies.^{10,13-20} However, it is still not clear whether human adipose-derived stem cells have the endothelial differentiation capacity that might be of clinical significance, under the same differentiation conditions.

Our aim in this study was to compare the endothelial differentiation capacities of rat and human adipose-derived stem cells using the same

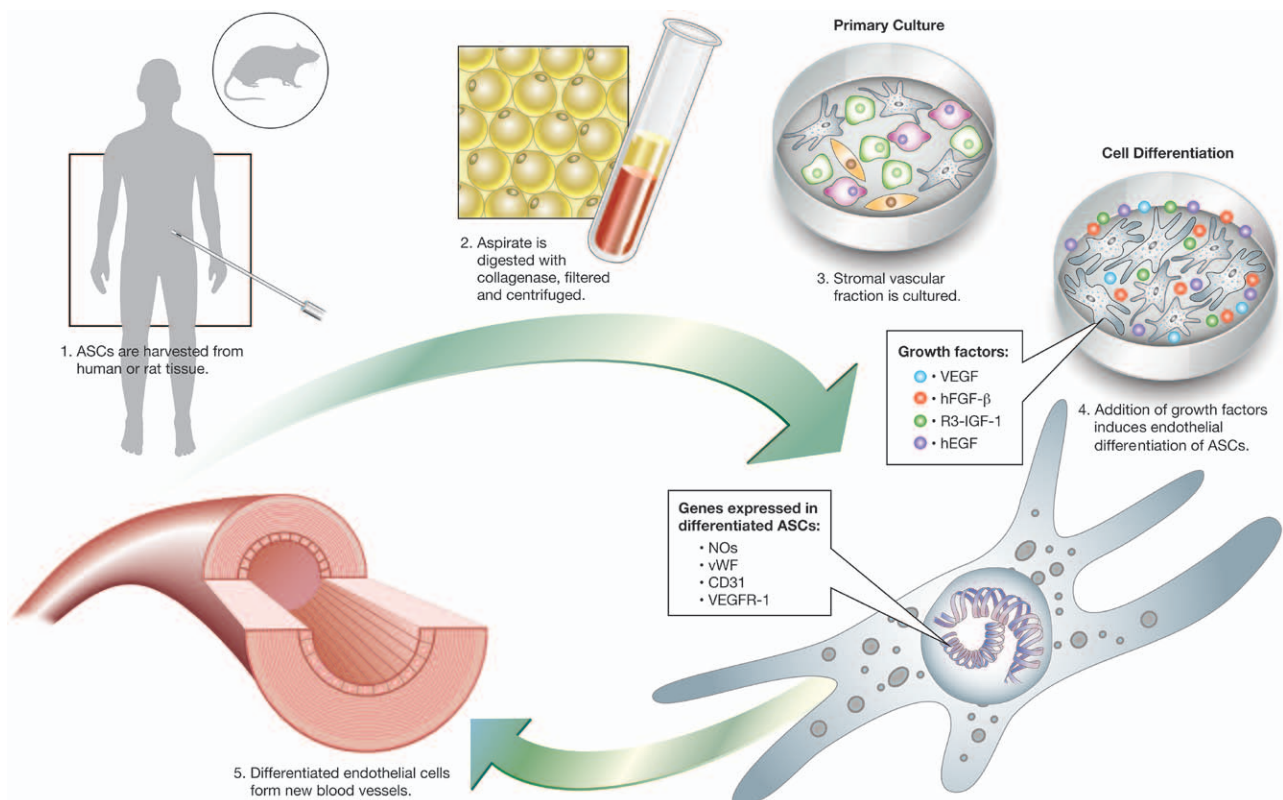


Fig. 1. The illustration outlines the experimental plan. We have harvested rat and human ASCs and differentiated these cells into endothelial cells. The newly obtained endothelial cells can eventually be used to form new blood vessels in tissue-engineered constructs.

differentiation medium and determine the clinical potential of human adipose-derived stem cells as a source for endothelial cells. We first harvested and grew adipose-derived stem cells from human and rat fat tissue, and then compared and contrasted the morphology, genetic profile, and functional status of differentiated cells over a time span of 3 weeks.

MATERIALS AND METHODS

Harvest and Characterization of Adipose-Derived Stem Cells

All animal experiments were approved by the University of California, Davis Institutional Animal Care and Use Committee (protocol no. FDG20110033A). Human adipose-derived stem cells were harvested from human adipose tissue specimens discarded during routine operations, and rat cells were harvested from the inguinal fat pads of Lewis rats. Briefly, adipose tissues were digested in 0.15% collagenase solution for 45 to 60 minutes. After digestion, the solutions were filtered through 100- μ m filters (Thermo Fisher Scientific, Pittsburgh, Pa.) and centrifuged at 1200 rpm for 5 minutes. After centrifugation, the supernatant was removed and the resultant cell pellet was washed with sterile phosphate-buffered saline to eliminate any contamination. Finally, the cells were plated onto 60-mm cell culture dishes (Corning). Human and rat adipose-derived stem cells were allowed to grow until 70 to 80 percent confluence in culture dishes. Fresh medium was added to dishes two times per week. Adipose-derived stem cells between passages III and V were used for the future experiments.

Adipose-derived stem cells were characterized by flow cytometry and trilineage differentiation.

Table 1. Content of EGM-2MV Endothelial Differentiation Medium

Medium	Volume (ml)
EBM 2 Basal Medium	500
Microvascular SingleQuots Kit	
VEGF	0.5
Fetal bovine serum	25
Hydrocortisone	0.2
hFGF-B	2.0
R3-IGF-1	0.5
Ascorbic acid	0.5
hEGF	0.5
GA-1000	0.5

hFGF- β , human fibroblast growth factor beta; R3-IGF-1, R3-insulin like growth factor-1; hEGF, human epidermal growth factor; GA-1000, gentamicin/amphotericin-B.

For flow cytometry, human cells in suspension were incubated with anti-CD24-PE, anti-CD44-FITC, anti-CD90-FITC, and anti-CD31-Alexa Fluor 647 (AF647) antibodies (Human Stem Cell Analysis Kit; BD Biosciences, San Diego, Calif.) in the dark, at room temperature, for 30 minutes. The cells were washed with wash buffer (0.5% fetal bovine serum in phosphate-buffered saline) and fixed in neutral 4% paraformaldehyde solution for 30 minutes. The cells were analyzed using a flow cytometer (BD Biosciences). Rat cells were characterized using anti-CD45-phycoerythrin (Biolegend, San Diego, Calif.), anti-CD44-phycoerythrin (eBioscience, San Diego, Calif.), anti-CD31-phycoerythrin (BD Biosciences), and anti-CD90-fluorescein isothiocyanate (EMD Millipore, Billerica, Mass.) antibodies.

To induce adipogenic, chondrogenic, and osteogenic differentiation, adipose-derived stem cells were cultured in StemPro Adipogenesis and Chondrogenesis media (Thermo Fisher Scientific) for 14 days, and Osteogenesis Media (Thermo Fisher Scientific) for 21 days. For chondrogenic differentiation, the micromass pellet method was used. Adipogenic, chondrogenic, and osteogenic differentiations were confirmed with Oil Red O, Alcian blue, and Alizarin red staining, respectively.

Endothelial Differentiation of Adipose-Derived Stem Cells

Once the cell population was adequate, human and rat adipose-derived stem cells were differentiated into endothelial cells according to previously described, widely established methods^{14,16,18} using the EGM-2MV Bullet Kit (Lonza Pharmaceuticals, Basel, Switzerland) containing the growth factors listed in Table 1. Fresh medium was added to cell cultures two times per week, and after 1, 2, and 3 weeks of differentiation, cells were harvested for evaluation with quantitative reverse-transcriptase polymerase chain reaction, flow cytometry, and three-dimensional angiogenic sprouting assays (Fig. 1).

Quantitative Reverse-Transcriptase Polymerase Chain Reaction

Differentiated cells were detached by trypsin (Thermo Fisher Scientific, Pittsburgh, Pa.) and total RNA was isolated from the cells using the RNeasy kit (Qiagen, Valencia, Calif.). cDNA was synthesized from RNA using a reverse transcription kit (Takara, Shiga, Japan). Afterward, cDNA was amplified using a quantitative reverse-transcriptase polymerase chain reaction machine

Table 2. Custom Primers Used for Quantitative Reverse-Transcriptase Polymerase Chain Reaction

	Forward	Reverse
Human		
<i>CD31</i>	5'-CACAGATGAGAACCACGCCT-3'	5'-GACCTCAATCTGAGCCCACC-3'
<i>VEGFR-1</i>	5'-AGAGGTGAGCACTGCAACAA-3'	5'-TAGCTGTGTGGTGCAGTCAG-3'
<i>NOs</i>	5'-CTGAAGGCTGGCATCTGGAA-3'	5'-CAGAGCTCGGTGATCTCCAC-3'
<i>vWF</i>	5'-ACACCTGCATTTGCCGAAAC-3'	5'-ATGCGGAGGTCACCTTTTCAG-3'
<i>GAPDH</i> *	5'-AATGGGCAGCCGTTAGGAAA-3'	5'-GCGCCCAATACGACCAAATC-3'
Rat		
<i>CD31</i>	5'-TCACCAAGAGAACGGAAGGC-3'	5'-TATTTGACGGCAGCAGAGGA-3'
<i>VEGFR-1</i>	5'-TCACCACGGACCTCAATAGA-3'	5'-CGATGCTTCACGCTGATAAA-3'
<i>NOs</i>	5'-GCTCCCAACTGGACCATCTC-3'	5'-TTTCAGAGAGGGTGGTTTCC-3'
<i>vWF</i>	5'-CCGAGCCATACCTGGACATC-3'	5'-CGGATGCGCTTCTGAGAGAT-3'
<i>GAPDH</i>	5'-AGACAGCCGCATTCCCTTGT-3'	5'-TGATGGCAACAATGTCAAGT-3'

*Housekeeping gene.

(Thermo Fisher Scientific). The fold changes in the expression levels of the endothelial cell-specific genes *CD31*, vascular endothelial growth factor receptor-1 (*VEGFR-1*), nitric oxide synthase (*NOs*), and von Willebrand factor (*vWF*), were calculated using the $\Delta\Delta Ct$ method (Fig. 1). The primers used for quantitative reverse-transcriptase polymerase chain reaction are listed in Table 2.

Flow Cytometry

Differentiated cells were trypsinized, washed, incubated with AF647-coupled anti-human CD31 antibody and phycoerythrin-coupled anti-rat CD31 antibody, and analyzed with a flow cytometer (BD LSRFortessa; BD Biosciences) at 1, 2, and 3 weeks.

Three-Dimensional Sprouting Assay

Three-dimensional sprouting assays were performed to examine the functional status of the differentiated adipose-derived stem cells. Cytodex 3 microcarrier beads (16.8 mg) (no. 17-0485-01; GE Healthcare, Buckinghamshire, United Kingdom) were hydrated in 10 ml of phosphate-buffered saline for at least 3 hours with gentle agitation. After sterilization by autoclaving, the beads were mixed with approximately 2×10^6 endothelial cell differentiated human or rat adipose-derived stem cells in 1.5 ml of warm EGM-2MV medium, transferred to a round-bottom flow cytometry tube (Thermo Fisher Scientific), and incubated at 37°C with gentle mixing by inverting the tube three to five times every 20 minutes for 4 hours. The cell-laden beads were then washed with EGM-2MV medium, transferred to T25 flasks, and cultured at 37°C on a rotating orbital shaker (approximately 60 rpm) with daily medium changes until the beads became confluent. The cell-laden beads were then fluorescently stained with Hoescht 33342 (Life Technologies, Carlsbad, Calif.)

and Calcein AM (Anaspec, Inc., Fremont, Calif.). For analysis of three-dimensional sprout formation, the cell-laden beads were incorporated within fibrin gels as described previously with minor modifications.²¹⁻²⁴ Briefly, beads suspended in EGM-2MV were combined with fibrinogen (Sigma, St Louis, Mo.) solution supplemented with aprotinin (Sigma) and placed in 24-well plates. A second solution containing thrombin was then added at a 4:5 ratio and the plates were incubated at 37°C for 30 minutes. The cross-linked gels were then topped with EGM-2MV and incubated at 37°C for 1 day. At this endpoint, the gels were washed with phosphate-buffered saline and fixed overnight at 4°C in 4% formaldehyde. The total number of beads, empty beads, and sprouts was manually quantified for each well. A sprout was identified as more than one cell migrating outward linearly while remaining anchored to the bead. The average number of sprouts per bead with cells was then reported per well ($n = 4$).

RESULTS

Harvest and Characterization of Adipose-Derived Stem Cells

Human and rat adipose-derived stem cells were positive for the mesenchymal stem cell markers CD90 and CD44, whereas human cells were negative for CD24 (lymphocyte marker) and rat cells were negative for CD45 (leukocyte marker). Human cells were negative for CD31 initial cultures; however, up to 8.65 percent of rat cells were CD31⁺ in initial cultures (Fig. 2). This was most likely because of the presence of CD31⁺ endothelial cells in the initial stromal vascular fraction harvested from fat tissue. However, mature endothelial cells and other cell types (e.g., white blood cells) in stromal vascular fraction are eventually eliminated

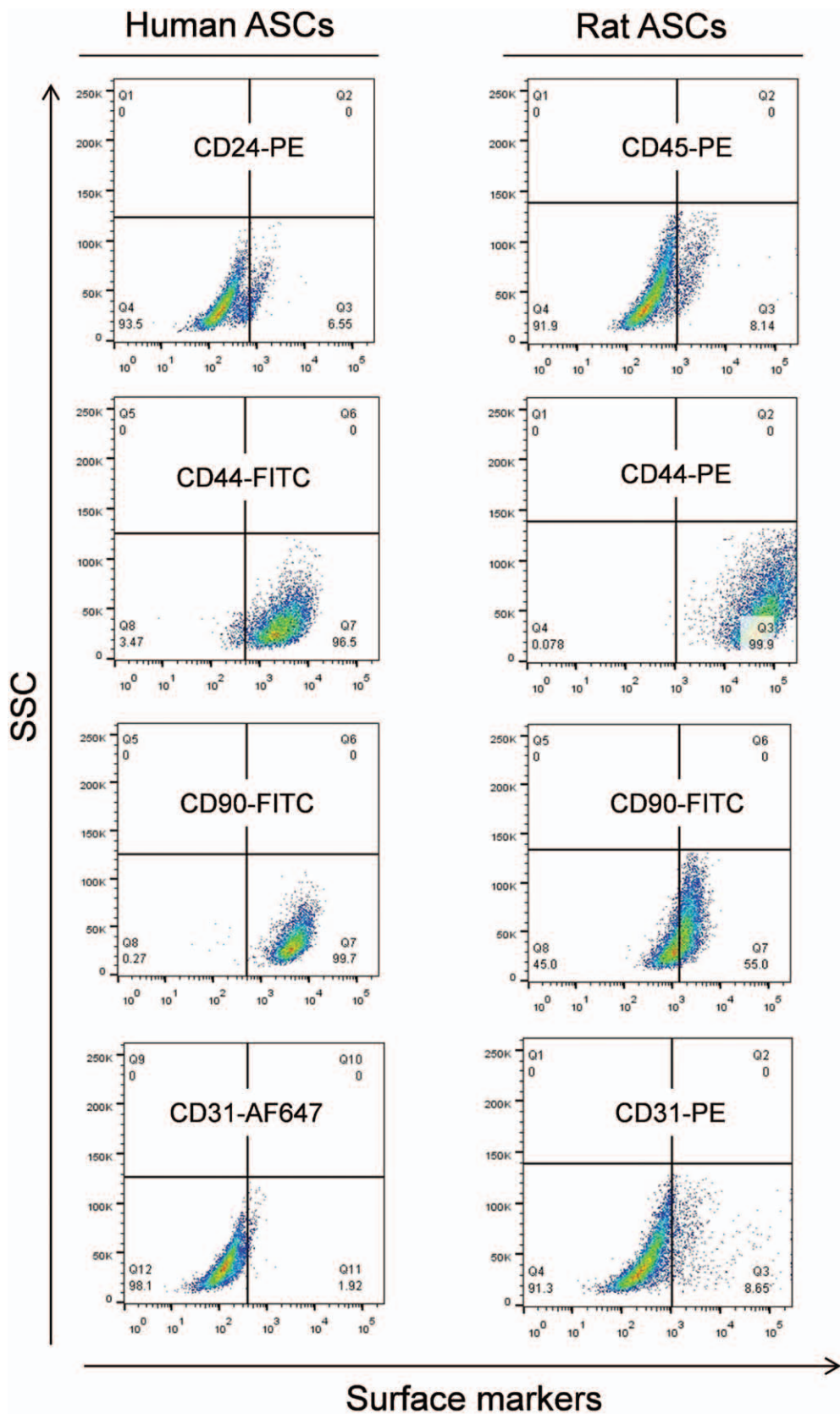


Fig. 2. Flow cytometric characterization of human and rat adipose-derived stem cells. Both cell types were positive for common mesenchymal stem cell markers (CD90 and CD44) and mostly negative for endothelial (CD31) and white blood cell markers (CD24 and CD45).

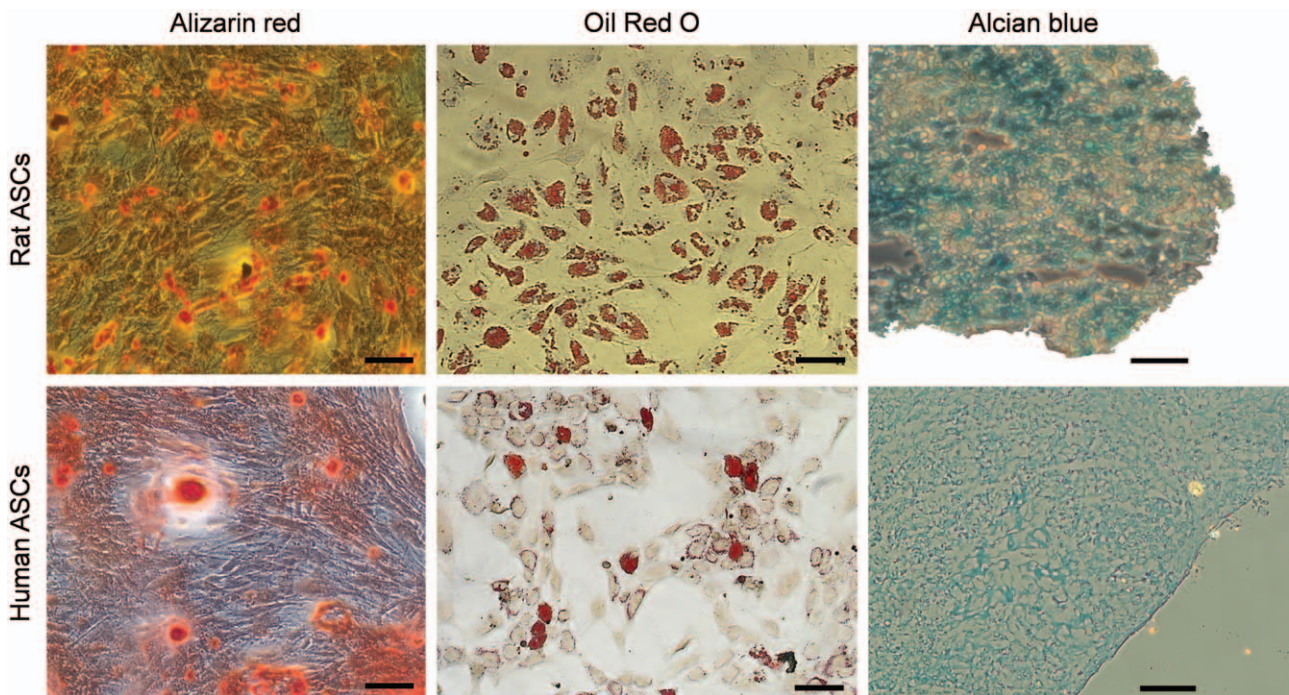


Fig. 3. Trilineage differentiation of rat adipose-derived stem cells (*above*) and human adipose-derived stem cells (*below, left and below, center*). (*Left*) Images showing the bony matrix secreted by differentiated rat and human adipose-derived stem cells stained red with Alizarin red. *Microbars* = 100 μ m. (*Center*) The lipid vacuoles within the cytoplasm of differentiated rat and human adipose-derived stem cells are visible with Oil Red O. *Microbars* = 100 μ m. (*Right*) The chondrogenic pellet was stained positive with Alcian blue staining. *Microbars* = 1 mm.

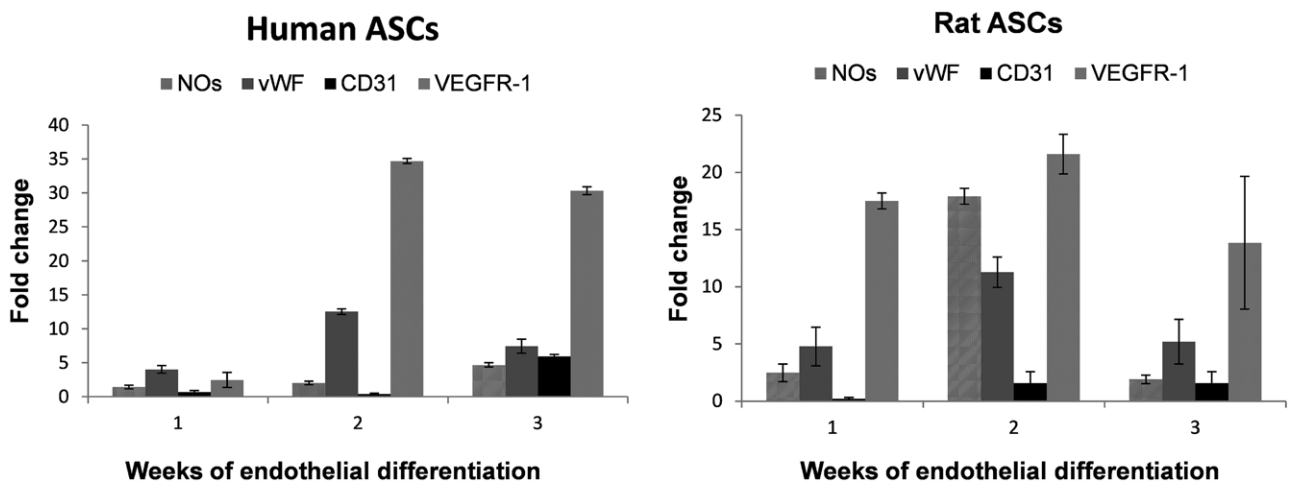


Fig. 4. The changes in expression levels of endothelial cell-specific genes were detected by quantitative reverse-transcriptase polymerase chain reaction in human (*left*) and rat (*right*) adipose-derived stem cell cultures during endothelial differentiation. The expression of the genes made a peak at the second week of differentiation in both cell cultures. ASCs, adipose-derived stem cells.

from the cell cultures. In our study, the percentage of CD31⁺ cells in rat adipose-derived stem cell cultures dropped to 0.5 percent 1 week after the beginning of differentiation. Adipose-derived stem cells were successfully differentiated into adipocytes, osteocytes, and chondrocytes, demonstrating their multipotent stem cell characteristics (Fig. 3).

Quantitative Reverse-Transcriptase Polymerase Chain Reaction

The expression of the *CD31* gene in human adipose-derived stem cells increased 0.7 ± 0.2 -, 0.4 ± 0.1 -, and 5.9 ± 0.2 -fold after 1, 2 and 3 weeks of differentiation, respectively (Fig. 4, *left*). The fold changes in expression of *VEGFR-1*, *NOs*, and *vWF*

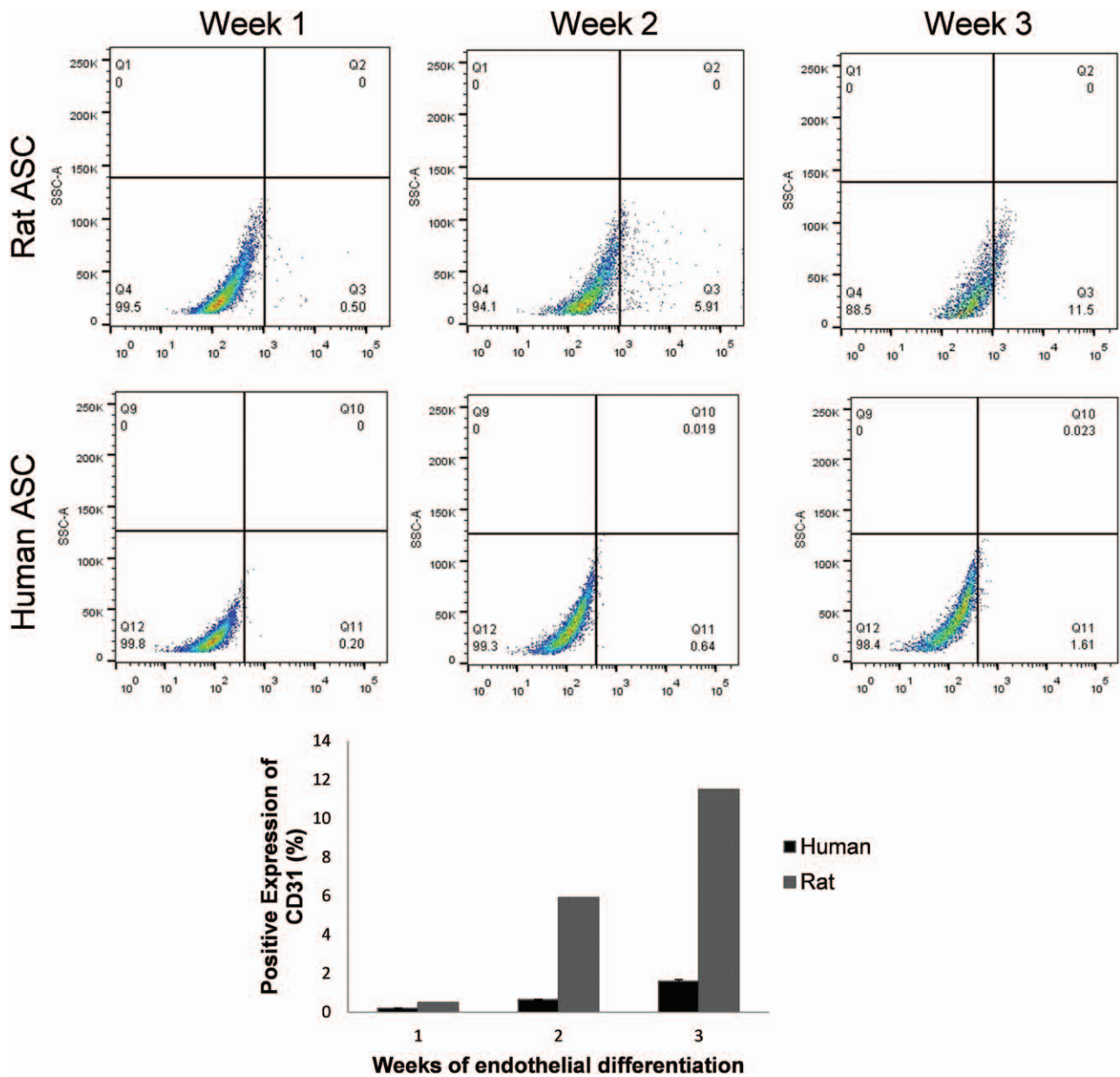


Fig. 5. (Above and center) The surface expression of CD31 detected by flow cytometry increased throughout the differentiation period in both rat and human adipose-derived stem cell (ASC) cultures. (Below) Graph showing the percentage of CD31⁺ cells in human and rat adipose-derived stem cells cultures at each time point.

genes in human adipose-derived stem cells were 2.4 ± 1.1 , 34.7 ± 0.3 , and 30.3 ± 0.5 ; 1.4 ± 0.27 , 2.03 ± 0.25 , and 4.6 ± 0.3 ; and 4.04 ± 0.5 , 12.5 ± 0.3 , and 7.4 ± 1.04 at weeks 1, 2, and 3, respectively (Fig. 4, left). The expression of the *CD31* gene increased 0.2 ± 0.1 -, 1.5 ± 1.01 -, and 1.5 ± 0.9 -fold in rat adipose-derived stem cells after 1, 2, and 3 weeks of differentiation, respectively. The fold changes in expression of *VEGFR-1*, *NOs*, and *vWF* genes in rat adipose-derived stem cells were 17.5 ± 0.6 , 21.6 ± 1.7 , and 13.8 ± 5.8 ; 2.4 ± 0.7 , 17.9 ± 0.6 , and 1.9 ± 0.3 ; and 4.7 ± 1.6 , 11.2 ± 1.3 , and

5.2 ± 1.9 at weeks 1, 2, and 3, respectively (Fig. 4, right).

Flow Cytometry

CD31 expression in rat adipose-derived stem cell cultures increased steadily from 0.5 percent at the first week of differentiation to 5.91 percent at the second week of differentiation and to 11.5 percent at the third week of differentiation (Fig. 5, above, and center). In human adipose-derived stem cell cultures, the percentage of cells expressing

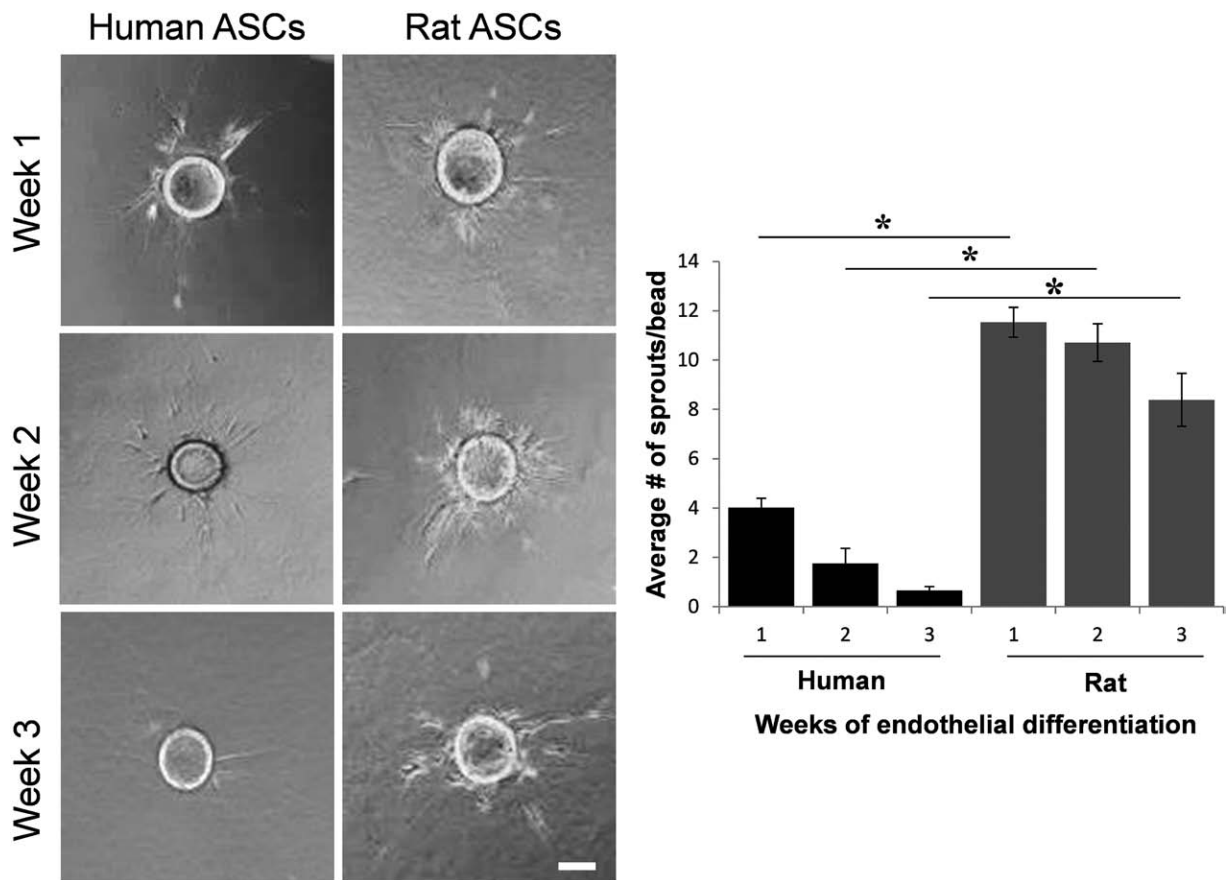


Fig. 6. (Left) Representative photomicrographs showing the vascular sprouts formed by endothelial cells derived from human and rat adipose-derived stem cells after 1, 2, and 3 weeks of differentiation. *Microbar* = 100 μ m. (Right) Graph showing the average number of sprouts formed by human and rat cells after 1, 2, and 3 weeks of endothelial differentiation (* p < 0.05).

CD31 was 0.2, 0.64, and 1.6 percent at weeks 1, 2, and 3 respectively (Fig. 5, *above*, and *center*). Even though these values were lower than in rat cell cultures, there was increasing CD31 expression as differentiation continued, similar to rat cell cultures.

Three-Dimensional Sprouting Assay

The average number of sprouts per bead formed by differentiated human and rat adipose-derived stem cells decreased as differentiation progressed (Fig. 6). In rat adipose-derived stem cell cultures, an average of 11.5 ± 0.6 , 10.7 ± 0.8 , and 8.4 ± 1.1 sprouts per bead were formed at weeks 1, 2, and 3 of differentiation, respectively. In human cultures, an average of 4.0 ± 0.4 , 1.8 ± 0.6 , and 0.7 ± 0.1 sprouts per bead were formed at weeks 1, 2, and 3 of differentiation, respectively (Fig. 6, *right*). The number of sprouts formed by rat adipose-derived stem cells was significantly higher than human adipose-derived stem cells at all time points (p < 0.05).

DISCUSSION

Endothelial differentiation capacity of adipose-derived stem cells is explained by the hypothesis that adipose-derived stem cells and endothelial cells share a common hematopoietic origin.^{25,26} In a study supporting this hypothesis, a CD34⁺/CD31⁻ subfraction of stromal vascular cells obtained from adipose tissue could be differentiated into endothelial cells, and potentially promoted neovascularization.^{14,27} This was seen as evidence of the hematopoietic origin of adipose-derived stem cells, just like endothelial cells, because CD34 is a hematopoietic-marker.¹⁴ In our study, we also demonstrated that human and rat adipose-derived stem cells acquire an endothelial phenotype and express endothelial markers *CD31*, *vWF*, *NOs*, and *VEGFR-1* (peak at 2 weeks) when cultured in EGM-2MV medium. The fold changes in the expression levels of these genes followed a very similar pattern in both cell types, wherein the *VEGFR-1* gene had the highest expression at

the second week of differentiation. The only difference was the expression of *NOs* that showed a steady increase in human adipose-derived stem cells during differentiation, whereas it made a significant peak at the second week in the rat group.

Despite the similarity in gene expression, CD31 expression on the surface of human adipose-derived stem cells was significantly lower than that of rat adipose-derived stem cells as detected by flow cytometry. This finding suggests the involvement of other pathways in endothelial differentiation of adipose-derived stem cells resulting in different cell surface protein expression in human and rat cells. Two of the previously studied genetic pathways taking part in endothelial differentiation of adipose-derived stem cells are global demethylation in CD31 and CD144 promoters,²⁸ and the PI3K pathway.²⁹ Further investigation of these pathways may give us a better understanding of the mechanisms underlying different levels of CD31 expression in human and rat adipose-derived stem cells in response to endothelial cell differentiation medium. Lower levels of CD31 expression in human adipose-derived stem cells can also be explained by the decreased effectiveness of the growth factors in EGM-2MV medium on human adipose-derived stem cells. Using completely human-originated growth factors or increasing VEGF concentration (up to 50 ng/ml) in EGM-2MV medium, as recommended before,²⁹ may increase the CD31 expression to levels observed in rat adipose-derived stem cells.

Limited increments in the expression of CD31 in adipose-derived stem cells exposed to endothelial differentiation medium has also been attributed to contamination of early adipose-derived stem cell cultures with endothelial cells or endothelial progenitor cells originating from adipose tissue, and selective proliferation of these cells with the effects of growth factors in the differentiation medium.^{12,28} However, we confirmed the absence of a significant endothelial cell contamination in our adipose-derived stem cell cultures by documenting the absence of CD31 expression before differentiation using flow cytometry. The CD31⁺ cells that were observed in initial rat cultures disappeared after 1 week of differentiation; therefore, it is unlikely that the rise in CD31 expression we observed in the following weeks was caused by the endothelial cell contamination.

Rat adipose-derived stem cells also formed significantly higher numbers of sprouts at all time points in comparison with human adipose-derived stem cells. However, in both cell types, sprout-forming capacity declined as differentiation progressed.

Therefore, there was an inverse correlation between the number of sprouts formed and CD31 expression. Given the central role of CD31 in new-vessel formation,^{30,31} a direct correlation between CD31 expression and sprout formation is expected. However, as documented previously, the lack of CD31 expression does not necessarily correlate with impaired vessel formation.³² Although there is no doubt that CD31 has a role in the vessel formation by endothelial cells, the functional status of cells is the result of a complex interaction between several factors, and it may be inaccurate to correlate the cellular functions with only one cellular marker.

Considered together, our results documented the endothelial differentiation potential of both human and rat adipose-derived stem cells. Nevertheless, the response of human adipose-derived stem cells to EGM-2MV endothelial differentiation medium was significantly lower than rat adipose-derived stem cells in terms of endothelial cell-specific gene and cell surface CD31 expression and sprout-forming capacity. This finding is not entirely surprising, as these cells originate from different species. The differences in the biology of rat and human adipose-derived stem cells is the most likely explanation for different behavior under the same circumstances, and makes a direct comparison between these two cell types difficult. However, in a preliminary experiment, we could also demonstrate that endothelial cells derived from rat adipose-derived stem cells could form more sprouts per bead in comparison with human microvascular endothelial cells that were used as a positive control. [See Figure, Supplemental Digital Content 1, which shows that endothelial cells (*ECs*) derived from human adipose-derived stem cells (*ADCs*) formed more sprouts compared with human microvascular endothelial cells (*HMVECs*) (**p* < 0.05), <http://links.lww.com/PRS/B932>.]

CONCLUSIONS

To establish human adipose-derived stem cells as a clinically significant source of endothelial cells, it might be necessary to modify the differentiation medium (concentrations of the growth factors, particularly VEGF) or increase the duration of differentiation. In addition, use of methods such as the application of shear stress,^{29,33,34} highly resistant thermoplastic sacrificial templates,³³ nanostructured surfaces,^{35,36} chemicals such as sphingosine-1-phosphate and leptin,^{22,37,38} and hypoxic preconditioning^{15,22} may further increase the endothelial differentiation capacity of human adipose-derived stem cells.

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REFERENCES

- Schäffler A, Büchler C. Concise review: Adipose tissue-derived stromal cells. Basic and clinical implications for novel cell-based therapies. *Stem Cells* 2007;25:818–827.
- Murphy SV, Atala A. Organ engineering: Combining stem cells, biomaterials, and bioreactors to produce bioengineered organs for transplantation. *Bioessays* 2013;35:163–172.
- Kannan RY, Salacinski HJ, Sales K, Butler P, Seifalian AM. The roles of tissue engineering and vascularisation in the development of micro-vascular networks: A review. *Biomaterials* 2005;26:1857–1875.
- Kim S, von Recum H. Endothelial stem cells and precursors for tissue engineering: Cell source, differentiation, selection, and application. *Tissue Eng Part B Rev*. 2008;14:133–147.
- Barrilleaux B, Phinney DG, Prockop DJ, O'Connor KC. Review: Ex vivo engineering of living tissues with adult stem cells. *Tissue Eng*. 2006;12:3007–3019.
- Orbay H, Tobita M, Mizuno H. Mesenchymal stem cells isolated from adipose and other tissues: Basic biological properties and clinical applications. *Stem Cells Int*. 2012;2012:461718.
- Herrmann M, Binder A, Menzel U, Zeiter S, Alini M, Verrier S. CD34/CD133 enriched bone marrow progenitor cells promote neovascularization of tissue engineered constructs in vivo. *Stem Cell Res*. 2014;13:465–477.
- Fu WL, Xiang Z, Huang FG, et al. Coculture of peripheral blood-derived mesenchymal stem cells and endothelial progenitor cells on strontium-doped calcium polyphosphate scaffolds to generate vascularized engineered bone. *Tissue Eng Part A* 2015;21:948–959.
- Dissanayaka WL, Hargreaves KM, Jin L, Samaranyake LP, Zhang C. The interplay of dental pulp stem cells and endothelial cells in an injectable peptide hydrogel on angiogenesis and pulp regeneration in vivo. *Tissue Eng Part A* 2015;21:550–563.
- DiMuzio P, Tulenko T. Tissue engineering applications to vascular bypass graft development: The use of adipose-derived stem cells. *J Vasc Surg*. 2007;45(Suppl A):A99–103.
- Zuk PA, Zhu M, Ashjian P, et al. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell* 2002;13:4279–4295.
- Zuk PA, Zhu M, Mizuno H, et al. Multilineage cells from human adipose tissue: Implications for cell-based therapies. *Tissue Eng*. 2001;7:211–228.
- Behr B, Tang C, Germann G, Longaker MT, Quarto N. Locally applied vascular endothelial growth factor A increases the osteogenic healing capacity of human adipose-derived stem cells by promoting osteogenic and endothelial differentiation. *Stem Cells* 2011;29:286–296.
- Planat-Benard V, Silvestre JS, Cousin B, et al. Plasticity of human adipose lineage cells toward endothelial cells: Physiological and therapeutic perspectives. *Circulation* 2004;109:656–663.
- Bekhte MM, Finkensieper A, Rebhan J, et al. Hypoxia, leptin, and vascular endothelial growth factor stimulate vascular endothelial cell differentiation of human adipose tissue-derived stem cells. *Stem Cells Dev*. 2014;23:333–351.
- Cao Y, Sun Z, Liao L, Meng Y, Han Q, Zhao RC. Human adipose tissue-derived stem cells differentiate into endothelial cells in vitro and improve postnatal neovascularization in vivo. *Biochem Biophys Res Commun*. 2005;332:370–379.
- Colazzo F, Alrashed F, Saratchandra P, et al. Shear stress and VEGF enhance endothelial differentiation of human adipose-derived stem cells. *Growth Factors* 2014;32:139–149.
- Colazzo F, Chester AH, Taylor PM, Yacoub MH. Induction of mesenchymal to endothelial transformation of adipose-derived stem cells. *J Heart Valve Dis*. 2010;19:736–744.
- Cornejo A, Sahar DE, Stephenson SM, et al. Effect of adipose tissue-derived osteogenic and endothelial cells on bone allograft osteogenesis and vascularization in critical-sized calvarial defects. *Tissue Eng Part A* 2012;18:1552–1561.
- Auxenfans C, Lequeux C, Perrusel E, Mojallal A, Kinikoglu B, Damour O. Adipose-derived stem cells (ASCs) as a source of endothelial cells in the reconstruction of endothelialized skin equivalents. *J Tissue Eng Regen Med*. 2012;6:512–518.
- Silva EA, Kim ES, Kong HJ, Mooney DJ. Material-based deployment enhances efficacy of endothelial progenitor cells. *Proc Natl Acad Sci USA* 2008;105:14347–14352.
- Williams PA, Stilhano RS, To VP, Tran L, Wong K, Silva EA. Hypoxia augments outgrowth endothelial cell (OEC) sprouting and directed migration in response to sphingosine-1-phosphate (S1P). *PLoS One* 2015;10:e0123437.
- Silva EA, Mooney DJ. Spatiotemporal control of vascular endothelial growth factor delivery from injectable hydrogels enhances angiogenesis. *J Thromb Haemost*. 2007;5:590–598.
- Silva EA, Eseonu C, Mooney DJ. Endothelial cells expressing low levels of CD143 (ACE) exhibit enhanced sprouting and potency in relieving tissue ischemia. *Angiogenesis* 2014;17:617–630.
- Katz AJ, Tholpady A, Tholpady SS, Shang H, Ogle RC. Cell surface and transcriptional characterization of human adipose-derived adherent stromal (hADAS) cells. *Stem Cells* 2005;23:412–423.
- Zannettino AC, Paton S, Arthur A, et al. Multipotential human adipose-derived stromal stem cells exhibit a perivascular phenotype in vitro and in vivo. *J Cell Physiol*. 2008;214:413–421.
- Urbich C, Dimmeler S. Endothelial progenitor cells functional characterization. *Trends Cardiovasc Med*. 2004;14:318–322.
- Boquest AC, Noer A, Sørensen AL, Vekterud K, Collas P. CpG methylation profiles of endothelial cell-specific gene promoter regions in adipose tissue stem cells suggest limited

- differentiation potential toward the endothelial cell lineage. *Stem Cells* 2007;25:852–861.
29. Zhang P, Moudgill N, Hager E, et al. Endothelial differentiation of adipose-derived stem cells from elderly patients with cardiovascular disease. *Stem Cells Dev.* 2011;20:977–988.
 30. Sauter B, Foedinger D, Sterniczy B, Wolff K, Rappersberger K. Immunoelectron microscopic characterization of human dermal lymphatic microvascular endothelial cells: Differential expression of CD31, CD34, and type IV collagen with lymphatic endothelial cells vs blood capillary endothelial cells in normal human skin, lymphangioma, and hemangioma in situ. *J Histochem Cytochem.* 1998;46:165–176.
 31. Matsumura T, Wolff K, Petzelbauer P. Endothelial cell tube formation depends on cadherin 5 and CD31 interactions with filamentous actin. *J Immunol.* 1997;158:3408–3416.
 32. Duncan GS, Andrew DP, Takimoto H, et al. Genetic evidence for functional redundancy of platelet/endothelial cell adhesion molecule-1 (PECAM-1): CD31-deficient mice reveal PECAM-1-dependent and PECAM-1-independent functions. *J Immunol.* 1999;162:3022–3030.
 33. Tocchio A, Tamplenizza M, Martello F, et al. Versatile fabrication of vascularizable scaffolds for large tissue engineering in bioreactor. *Biomaterials* 2015;45:124–131.
 34. Yamamoto K, Takahashi T, Asahara T, et al. Proliferation, differentiation, and tube formation by endothelial progenitor cells in response to shear stress. *J Appl Physiol (1985)* 2003;95:2081–2088.
 35. Shi Z, Neoh KG, Kang ET. In vitro endothelialization of cobalt chromium alloys with micro/nanostructures using adipose-derived stem cells. *J Mater Sci Mater Med.* 2013;24:1067–1077.
 36. Shi Z, Neoh KG, Kang ET, Poh CK, Wang W. Enhanced endothelial differentiation of adipose-derived stem cells by substrate nanotopography. *J Tissue Eng Regen Med.* 2014;8:50–58.
 37. Wu X, Rabkin-Aikawa E, Guleserian KJ, et al. Tissue-engineered microvessels on three-dimensional biodegradable scaffolds using human endothelial progenitor cells. *Am J Physiol Heart Circ Physiol.* 2004;287:H480–H487.
 38. Arya D, Chang S, DiMuzio P, Carpenter J, Tulenko TN. Sphingosine-1-phosphate promotes the differentiation of adipose-derived stem cells into endothelial nitric oxide synthase (eNOS) expressing endothelial-like cells. *J Biomed Sci.* 2014;21:55.

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