Published in final edited form as:
J Surg Res. 2010 October ; 163(2): e105–e112. doi:10.1016/j.jss.2010.04.025.

Availability of adipose-derived stem cells in patients undergoing vascular surgical procedures

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Abstract

Background—Most research evaluating adipose-derived stem cells (ASC) uses tissue obtained from young, healthy patients undergoing plastic surgical procedures. Given the propensity of other adult stem cell lines to diminish with increasing patient age and co-morbidities, we assess the availability of ASC in elderly patients undergoing vascular surgical procedures, and evaluate their acquisition of endothelial cell (EC) traits to define their potential use in vascular tissue engineering.

Methods and Methods—Adipose tissue obtained by liposuction from patients undergoing vascular procedures (n=50) was digested with collagenase and centrifuged to remove mature adipocytes. The resultant number of cells, defined as the stromal-vascular (SV) pellet, was quantified. Following a seven day culture period and negative selection for CD31 and CD45, the resultant number of ASC was quantified. After culture in differentiating media (EMG-2), ASCs were tested for the acquisition of endothelial-specific traits (expression of CD31, re-alignment in shear, cord formation on Matrigel).

Results—The SV pellet contained $2.87\pm0.34 \times 10^5$ cells/gm fat, and the resultant number of ASCs obtained was $1.41\pm0.18 \times 10^5$ cells/gm fat. Flow cytometry revealed a homogeneous ASC population (>98% positive for CD13, 29, 90). Advanced age or co-morbidity (obesity, diabetes, renal or peripheral vascular disease) did not significantly alter yield of ASC. After culture in differentiating media (EMG-2), ASCs acquired each of the endothelial-specific traits.

Conclusion—ASC isolation appears independent of age and co-morbidities, and ASCs harvested from patients with vascular disease retain their ability to differentiate into endothelial-like cells. Adipose tissue, therefore, is a practical source of autologous, adult stem cells for vascular tissue engineering.

Keywords

adult stem cells; adipose tissue; peripheral vascular disease

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DISCLOSURES None.

INTRODUCTION

As the prevalence of vascular disease continues to increase, the need for a suitable arterial replacement is clear. Vascular tissue engineering has emerged as a potential alternative to the problem of the currently available suboptimal bypass conduits. Tissue-engineering strategies for creation of an alternative conduit typically involve implanting synthetic or biological scaffolds with vascular cells. The use of autologous mature endothelial cells (EC) to line the lumen of a graft is limited by low numbers of cells obtained during harvest and slow expansion rate, requiring that large numbers of ECs be harvested for therapeutic use.[1,2] As a result, researchers have turned toward autologous adult stem cells as an alternative source of ECs. The majority of tissue engineering strategies have used adult stem cells harvested from bone marrow or endothelial progenitor cells (EPCs) harvested from blood.[3-7] While both of these stem cell populations have shown potential to differentiate into ECs, the availability of these cells is limited by advanced patient age and the presence of comorbid conditions associated with vascular disease.[2-4,8].

To evade the problem of stem cell availability, we have turned to adipose tissue as an abundant source of adult stem cells. Autologous adipose tissue is easily obtainable via liposuction aspiration with minimal patient morbidity. After processing and a brief culture period, the result is a large number of stem cells with homogeneous surface markers.[9,10] Prior work in our laboratory has shown that these adipose-derived stem cells (ASCs) have the potential to differentiate toward an EC lineage when exposed to chemical (growth factors) and mechanical (shear stress) stimuli as evidenced by (1) the expression of the endothelial cell marker CD31 and (2) morphologic changes (alignment in the direction of flow and cord formation after seeding).[2,11] In addition, we have shown that these differentiated ASC will establish a monolayer of cells on the graft lumen after seeding and flow conditioning.[12] Others have also confirmed the potential for ASCs to differentiate into ECs by documenting expression of endothelial markers, cord formation after seeding, and participation in neovascularization in animal models.[13-16] Most of the initial research using ASCs has been from specimens obtained from young, healthy patients undergoing plastic surgery.[9,13-16] Thus, the applicability of these studies in the vascular disease population is unknown, as it is possible that advanced age and comorbidities may affect the utility of ASCs.

The purpose of this study was (1) to determine whether ASC isolation is hindered by advanced age and comorbidities, and (2) to investigate if ASCs from patients with advanced age and comorbidities retain the potential to differentiate into an EC-like phenotype. Thus, our overall goal was to assess the utility of harvesting ASCs from the patient population most likely to benefit from this technology—the elderly patient with vascular disease.

METHODS

The following studies were approved by the Investigational Review Board of Thomas Jefferson University. All patients gave informed consent prior to tissue donation.

Isolation of ASC from human subcutaneous fat

Human adipose tissue samples were obtained from patients undergoing various elective vascular procedures at Thomas Jefferson University Hospital (Philadelphia, PA). Under sterile conditions, approximately 60 cc of tumescent solution (30mL 1% lidocaine, 30mL 0.5% bupivicaine, 10mL 4.2% sodium bicarbonate, 1mg epinephrine in 1 liter injectable normal saline) was infiltrated into the peri-umbilical region. A Mercedes 3mm × 9cm Aspiration Luer Lock Cannula (Byron Medical, Tucson, AZ) was used to withdraw the liposuction aspirate. The specimen was immediately placed on ice for transportation to the laboratory. The lipoaspirate was washed with PBS to remove all anesthetic and blood contamination followed

by filtration through a 250 μm sieve. The adipose tissue was digested with Collagenase I (1mg/ml; Worthington Biochemical Corp., Lakewood, NJ) for 1h at 37°C with gentle agitation. The slurry was centrifuged for 10 min at 1500 × g and the supernatant containing the mature adipocytes was discarded. The resulting stromal vascular (SV) pellet was washed with 0.1% BSA (Sigma-Aldrich, St. Louis, MO) and suspended in storage media (M-199 (Mediatech, Herndon, VA), fetal bovine serum (12.8%, Gemini BioProducts, Herndon, VA,) HEPES (1M, Fisher Biotech, Fair Lawn, NJ), heparin (Elkins-Sinn, Inc, Cherry Hill, NJ), antibiotic-antimycotic solution (100x solution; 10,000 U/ml Penicillin G, 25 μg/ml Amphotericin B, 10,000 μg/ml Streptomycin, Mediatech, Herndon, VA)), counted (Coulter Z-Series Counter), and plated onto gelatinized tissue culture flasks and incubated (37°C, 5% CO_2). The flasks were washed at 24 hours to remove all non-adherent cells.

Magnetic cell sorting of initial isolation

After approximately one week in culture, the adherent cells underwent negative selection using magnetic beads (MACS®; Miltenyi Biotec, Auburn, CA) to remove CD31+ (endothelial) and CD45+ (mononuclear) cells. Cells were released from the culture dish using trypsin and centrifuged at 300 × g. The resultant pellet was suspended in MACS® buffer (2mM EDTA and 0.5% bovine serum albumin), mixed with CD45-tagged microbeads and incubated at 4°C for 15 minutes. The cells were centrifuged, resuspended in MACS® buffer and passed through an LS Separation Column mounted in the Quadro- MACS® separator magnetic unit. The CD45-tagged cells were released from the separation column and counted (Coulter Z-Series Counter). The effluent underwent repeat procedure using CD31-tagged microbeads. The resultant number of flow through cells (CD45-, CD31-), defining the ASC population, were counted and re-plated onto tissue culture flasks. The medium of the subsequent cultures was changed three times weekly until near confluence, at which time the flasks were split 1:3.

Fluorescent Activated Cell Sorting (FACS) analysis

To assess the effectiveness of the negative selection, the stem cell isolates underwent FACS analysis for CD 13, 29, and 90, known markers associated with ASC.[10] The cells were released from culture with trypsin and centrifuged at 300 × g. The resultant cell pellet was washed and resuspended in 0.1% BSA. Individually, primary antibody (anti-human CD13-APC, anti-human CD29-PECY5, and anti-human CD90-FITC; BD Pharmigen, San Jose, CA) was added and incubated for 20 min at 37°C, 5% CO2. Samples were analyzed using a FACSaria™ Flow Cytometer (BD Biosciences, Franklin Lakes, NJ). Dermal microvascular endothelial cells (CADMEC; Cell Applications, San Diego, CA) were cultured similarly to the stem cells for use as a positive control, and unstained ASC were utilized as the negative control.

Endothelial Cell Differentiation

Randomly selected patient samples (n=15) were used to assess endothelial cell differentiation, but without a formal randomization process. Over the course of accruing the 50 patients in this study, the ASC were submitted for experimentation within several studies (differentiation, seeding of grafts, hypoxia studies, etc.) occurring within our laboratory. Submission into these experiments was on an ad hoc basis, depending upon the needs of the individual investigators within the lab without any regard as to the origin of the cell lines.

Following the negative selection for CD31 and CD45 cells, the resultant ASC were differentiated toward endothelial-like cells by culture in EGM-2 medium (Cambrex, East Rutherford, NJ) for two weeks. The medium was changed twice weekly and cells split when 80% confluent 1:3. Subsequent differentiation was evaluated by examining: 1) expression of the endothelial cell marker CD31 using RT-PCR, 2) cell alignment in the direction of fluid shear stress, and 3) cord formation upon plating on Matrigel (BD Biosciences, San Jose, CA).

RT-PCR—Total RNA from differentiated cells was extracted using RNeasy mini columns (QIAGEN, Valencia, CA). RNA concentration was obtained via spectrophotometer analysis. Reverse transcription was then performed by using the Promega Reverse Transcription System (Promega, Madison, WI). One microgram of total RNA was used in each reaction. The following primer pairs (Operon, Huntsville, AL) were used: 1) CD31 (Forward 5'-CACAGCAATTCCTCAGGCTA-3' Reverse 5'-TTCAGCCTTCAGCATGGTAG-3'), and 2) GAPDH (Forward 5'-AAGGTCGGAGTCAACGGATTTGGT-3' Reverse 5'-ACAAAGTGGTCGTTGAGGGCAATG-3'). Gel electrophoresis was then performed on a 2% agarose gel treated with ethidium bromide and visualized using a UV light box.

Shear stress experiments—Differentiated ASC were exposed to fluid shear stress using an orbital shear model as previously reported by Dardik et al.[17] Cells were plated onto gelatincoated 6-well plates and allowed to adhere for a period of 24 hours. The 6-well plate was then affixed to the surface of an orbital shaker (Bellco Biotechnology, Vineland, NJ) located within an incubator at 37 °C and 5% CO2. The plate was then rotated at 210 cycles/min, resulting in an average of 12 dynes at the periphery of the well.[17] After 72 hours, the 6-well plate was removed and photographs of the cells at the well periphery were taken using a phase contrast microscope.

Matrigel experiments—A 24-well plate was chilled at 4°C for 30 minutes after which 100 μL Matrigel was placed into each well. The plate was then placed in an incubator maintained at 37°C and 5% CO2 for an additional 30 minutes. Subsequently 100,000 cells were plated evenly on the Matrigel. After a 24 hour incubation period, the cultures were evaluated for cord formation using a phase contrast microscope.

Statistical Analysis

All quantified values (cell numbers) are expressed as mean ± standard deviation. Statistical analysis was performed using a two-tailed Student's t-test, with $P < 0.05$ considered significant.

RESULTS

Patient Characteristics

The demographic data from all 50 patients donating adipose tissue during elective vascular surgery are listed in Table 1. For the purposes of analysis, the patients were grouped by gender, age (>70 years being defined as elderly), obesity (BMI>30), presence of diabetes (defined as a pre-existing diagnosis of diabetes and/or the use of diabetic medications at home) and end-stage renal disease (defined by the need for dialysis) and peripheral vascular disease (defined as a patient undergoing intervention for arterial disease, either occlusive or aneurismal).

Adipose Tissue Isolation Produces a Homogeneous Population of Stem Cells

Per protocol, an average of 14 ± 0.8 gm of peri-umbilical adipose tissue was obtained from each patient via liposuction aspiration. After enzymatic dispersion, the resultant SV pellets contained $2.87 \pm 0.34 \times 10^5$ total cells per gm of fat. The initial cell isolates were cultured for one week in non-differentiating medium, after which they underwent negative selection for CD31 and CD45 to remove persistent co-cultured endothelial and monocyte/macrophage cell lines, respectively. An average of $7.4 \pm 1.3 \times 10^3$ CD31+ cells and $1.67 \pm 0.17 \times 10^4$ CD45+ cells per gram of fat were removed during the negative selection process. The resultant CD31-45- cell line defined the ASC population. An average of $1.41 \pm 0.18 \times 10^5$ ASC per gram of fat were obtained from each patient sample. FACS analysis of the ASC population was performed on 14 cells lines and revealed a homogeneous population of stem cells >98% positive for CD13, 29, and 90.

Patient Age and Co-morbidity Does Not Affect Cell Harvest or ASC Isolation

The total number of cells obtained in the SV pellet per gm of adipose tissue and the number of stem cells obtained per gm of adipose tissue were analyzed with respect to patient gender, patient age, BMI, DM, ESRD, and PVD. No significant differences were noted for gender, age, BMI, DM, ESRD, or PVD with respect to SV pellet harvest or ASC isolation (Figure 1). Several trends were noted, however. Males tended to yield a smaller SV pellet, but the ultimate ASC isolation appeared independent of gender. The presence of diabetes tended to decrease the average amount of both the SV pellet and ASC. Notably, the presence of PVD (occlusive plus aneurysm patients) tended to increase the yield of ASC.

ASCs from patients with advanced age and co-morbidity acquire EC characteristics

ASC from all randomly selected cells lines tested (n=15; of the 15, 10 were male, 11 elderly, 6 obese, 5 diabetic, 2 end-stage renal disease, 11 PVD) differentiated toward an EC lineage (Figure 2). Prior to differentiation, ASC did not express CD31, align in the direction of shear force, or form cords after seeding onto Matrigel. After culture for two weeks in differentiating medium (EGM-2), the ASC demonstrated each of these endothelial phenotypes.

Following culture in EGM-2 for two weeks, the stem cell markers CD13, 29 and 90 were re-evaluated by FACS in two patient cells lines. The average expression of each marker was noted to be 92%, 93%, and 67%, respectively.

DISCUSSION

Adult, autologous stem cells are an attractive source of cells for use as endothelial cell substitutes in cardiovascular tissue engineering strategies. The present study evaluates, for the first time, both the availability of adipose-derived stem cells and their ability to acquire endothelial cell traits in an elderly patient population with vascular disease. Given that much of the available information on this type of adult stem cell is derived from experiments using fat obtained from young and presumably healthy patients undergoing liposuction for cosmetic purposes, an important feature of this study is that it directly involves the patient population most likely to benefit from its results. We found that isolation of adipose tissue from these patients resulted in a homogeneous stem cell population, positive for markers CD13, 29 and 90. Importantly, gender or the presence of advanced age, obesity, diabetes, end-stage renal or peripheral vascular disease did not significantly diminish stem cell availability. Further, the stem cells taken from this patient population consistently acquired an endothelial cell-like phenotype after culture in differentiating medium as evidenced by expression of CD31, alignment in the direction of shear force, and cord formation upon seeding on Matrigel.

Our experience with ASC availability in patients with advanced age and multiple comorbidities differs from that of adult stem cells obtained from other sources. Advanced age and comorbidities are known to have a negative impact on both the number and function of adult mesenchymal stem cells from a variety of sources. The number of osteogenic progenitors in bone marrow has been shown to be significantly decreased in older patients.[18,19] D'Ippolito et al showed that the availability of these precursors decreased from 66.2 ± 9.6 per 10^6 cells for patients younger than age 40 to 14.7 ± 2.6 per 10^6 cells in patients of advanced age.[18] Similarly, other studies have reported a decline in both the number and proliferative capacity of muscle satellite cells with increased age.[20,21] Endothelial progenitor cells obtained from blood are also affected by age and comorbidities.[8] Advanced age, coronary artery disease, congestive heart failure, atherosclerosis, DM and chronic renal failure have all been shown to decrease both EPC availability and function.[8,22-24] This effect of age and age-related comorbidities on blood- and bone marrow-derived stem cells limits the therapeutic use of these stem cells in the patient population most likely to benefit from stem cell technologies. Our

results show that this is not the case for ASCs. Advanced age and comorbidities do not impact significantly the availability or function of ASCs, making elderly patients with vascular disease appropriate stem cell donors for therapeutic use.

An important aspect to consider when discussing the availability of stem cells is their potential for use in tissue engineering applications. Production of a tissue engineered vascular graft requires that an ample amount of stem cells be available in a relatively short period of time. The findings in this study suggest that ASCs have the potential for several different therapeutic uses. As determined from the number of ASCs obtained at one week after harvest, approximately 10 gms of adipose tissue would be required to confluently seed the luminal surface of a 10-cm × 4-mm bypass graft, a size appropriate for use in coronary artery bypass. Approximately 22 gms of adipose tissue would produce enough stem cells at one week to confluently seed the luminal surface of a 20-cm × 5-mm graft which is adequate for use as an arteriovenous graft for hemodialysis access. Similarly, 53 gms of adipose tissue would produce enough stem cells to confluently line a 40-cm × 6-mm bypass graft for use in lower extremity arterial bypass. Thus, approximately 10 to 50 gms of adipose tissue will provide enough stem cells to create a tissue engineered graft in a relatively short period of time. This quantity of adipose tissue is a reasonable size to be harvested from patients with vascular disease, making adipose tissue a practical source of autologous stem cells for tissue engineering applications in this patient population.

The ability to acquire characteristics of ECs is another important aspect of a stem cell's usefulness in tissue engineering. Endothelial cells have traditionally been used to line the luminal surface of a vascular graft in order to impart nonthrombogenicity. The use of native ECs is limited by low numbers obtained at harvest and a slow expansion rate, making this cell source somewhat impractical for use in tissue engineering.[1,2] Adipose-derived stem cells obtained from young, healthy patients undergoing elective plastic surgery have been shown to differentiate toward an EC phenotype[9,13-16] Until now, the utility of ASCs from the vascular disease population was unknown. This study showed that ASC obtained from patients with advanced age and comorbidities retained the ability to differentiate toward an EC phenotype. After differentiation in growth factor enriched medium (EGM-2), all samples subsequently expressed CD31, aligned in the direction of flow, and formed cords on Matrigel. We recognize that these three parameters do not represent an extensive evaluation of the ability of the stem cells from an elderly population to commit to an endothelial lineage; such a study is underway in our laboratory and is beyond the scope of the current investigation. None-the-less, these findings confirm the utility of ASCs from patients with vascular disease in tissue engineering applications.

The isolation procedure of adipose tissue first results in production of the stromal vascular pellet. The SV pellet contains a host of cells, including stem cells, pre-adipocytes, pericytes, monocytes, macrophages, and capillary endothelial cells. During magnetic cell sorting, the CD31 positive (endothelial cells) and CD45 positive (monocytes, macrophages) cells are removed, resulting in a homogenous stem cell population. Williams et al used liposuctionderived adipose tissue to obtain the microvascular CD31 (endothelial cell) population.[25] They showed that an average of 1×10^6 microvessel endothelial cells per gm of adipose was obtained and could be subsequently used to establish EC monolayers on vascular grafts. In our study, an average of $7.4 \pm 1.3 \times 10^3$ CD31 positive cells per gm of adipose were obtained at one week after harvest. This number of cells is not sufficient for use in tissue engineering. Therefore, our adipose samples cannot be used as a source of autologous, differentiated ECs using this technique. It is the stem cell population ($1.41 \pm 0.18 \times 10^5$ ASCs per gm of adipose) that provides an abundant source of cells for use in tissue engineering.

We have demonstrated that the isolation technique used in this study yields a population of mesenchymal stem cells which is homogeneous with respect to the markers CD13, 29 and 90. Although the results are preliminary and not statistically significant (n=2 cell lines), it appears that there is a down-regulation of these markers after differentiation in EGM-2 medium for two weeks. Although not clinically relevant, this result further supports the notion of loss of stemness by the cells and acquisition of alternative, in this case, endothelial markers.

The present study is limited by several factors. While we did observe differentiation of ASCs toward an EC phenotype, we did not quantify this differentiation with respect to patient demographics. It is possible that certain demographic factors may impact the ability of ASCs to differentiate toward ECs. However, all cell lines tested for differentiation potential showed the presence of all three tested EC characteristics. Additionally, we recognize that ascribing patients to various groups (PVD vs. non-PVD, for example) can be problematic, as frequently patients may be asymptomatic. None the less, the data clearly demonstrates the availability and EC differentiation capacity in the elderly population undergoing elective operations for PVD.

The trend towards a decreased availability in the diabetic population appears interesting. For other stem cell populations such as those derived from bone marrow, the decreased availability with advanced age and co-morbidity likely is due to the cells being used up over time. Given the stem cell population appears stable over a patient's lifetime, the trend noted in diabetics may be due to other factors. Diabetes is well-known to be associated with obesity, and the structure and function of adipose tissue in diabetes differs from the non-diabetic state.[26-28] We hypothesize that chronic glycosylation of the ASC surface proteins may decrease the ability of collagenase to release the cells during isolation. Alternatively, this stem cell population may not replenish well in diabetics. The decrease in ASC isolated from diabetic patients notwithstanding, construction of tissue-engineered grafts in this population remains very practical.

We recognize there are several limitations to the present study. In evaluating the availability of stem cells, other patient characteristics have been demonstrated to affect outcome including medications and other diseases such as the presence of coronary artery disease. Given patients with the later disease are also likely to benefit from adult stem cell-based therapies, future prospective evaluation of ASC in this population will be important. Further, this study was not designed to represent an exhaustive evaluation of the ability of these cells to differentiate into endothelial cells; rather, a screen of 15 patients was performed and noted the acquisition of three traits in all. We recognize that this does not prove the ability of ASC from this population to acquire important EC phenotypes, such as provision of a non-thrombogenic vascular lumen. The later will require extensive, in vivo investigation.

In summary, these results suggest that: 1) human adipose-derived stem cells can be isolated in abundance from abdominal wall fat, 2) adipose-derived stem cell availability is independent of age and co-morbidity, and 3) adipose-derived stem cells obtained from patients with vascular disease are capable of differentiating toward an endothelial cell lineage. These data indicate that adipose tissue is a viable source of autologous adult stem cells for use in the creation of a tissue engineered bypass graft in elderly patients with vascular disease. This is especially important in view of the recent finding the ASCs can be differentiated to induced pluripotent stem cells (iPS), thus raising still further the potential for ASCs in a wide variety of autologous stem cell applications in regenerative medicine.[29]

Acknowledgments

We thank Charles J. Yeo, M.D. for his critical review of the manuscript.

FUNDING SOURCES This work was supported by the following grants: NIH K08 HL076300-01 (PJD, TNT), the American Vascular Association (PJD), and the American Heart Association (PJD).

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Patient Characteristics	SVP/g	ASC/g
Gender		
Male	225,506 ± 24,687	125,029 ± 14,499
Female	395,594 ± 78,884	166,932 ± 42,468
P-value	0.053	0.36
Age (yrs)		
≥70	275,173 ± 72,532	168,344 ± 38,331
<70	313,722 ± 38,017	128,015 ± 19,545
P-value	0.642	0.359
BMI		
≥30	304,784 ± 51.621	148,578 ± 31,313
<30	274,707 ± 45,712	134,471 ± 21,583
P-value	0.665	0.713
DM		
Yes	212,064 ± 35,979	105,257 ± 12,304
No	328,742 ± 48,018	159,721 ± 26,562
P-value	0.058	0.07
ESRD		
Yes	314,151 ± 140,093	170,768 ± 67,975
No	281,516 ± 31,726	134,275 ± 17,275
P-value	0.83	0.62
PVD		
Yes	271,646 ± 40,014	147,248 ± 21,450
No	251,515 ± 60,294	81,844 ± 14,857
P-value	0.78	0.12

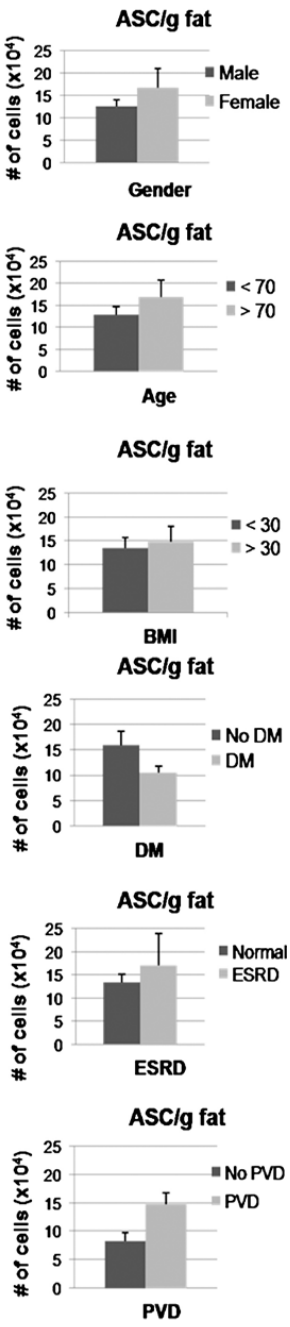

Figure 1. Association between patient characteristics and stem cell harvest

Univariate analysis was used to determine if various patient demographics affected the yield (as measured by number of cells obtained per gram of fat initially harvested) of the stromal vascular fraction (table) or stem cells (table, bar graph). Mean values are as listed ± standard error. With regards to the ASC, while no significant values were noted amongst the sample of 50 patients, diabetes tended to decrease the ASC yield while peripheral vascular disease tended to increase the yield.

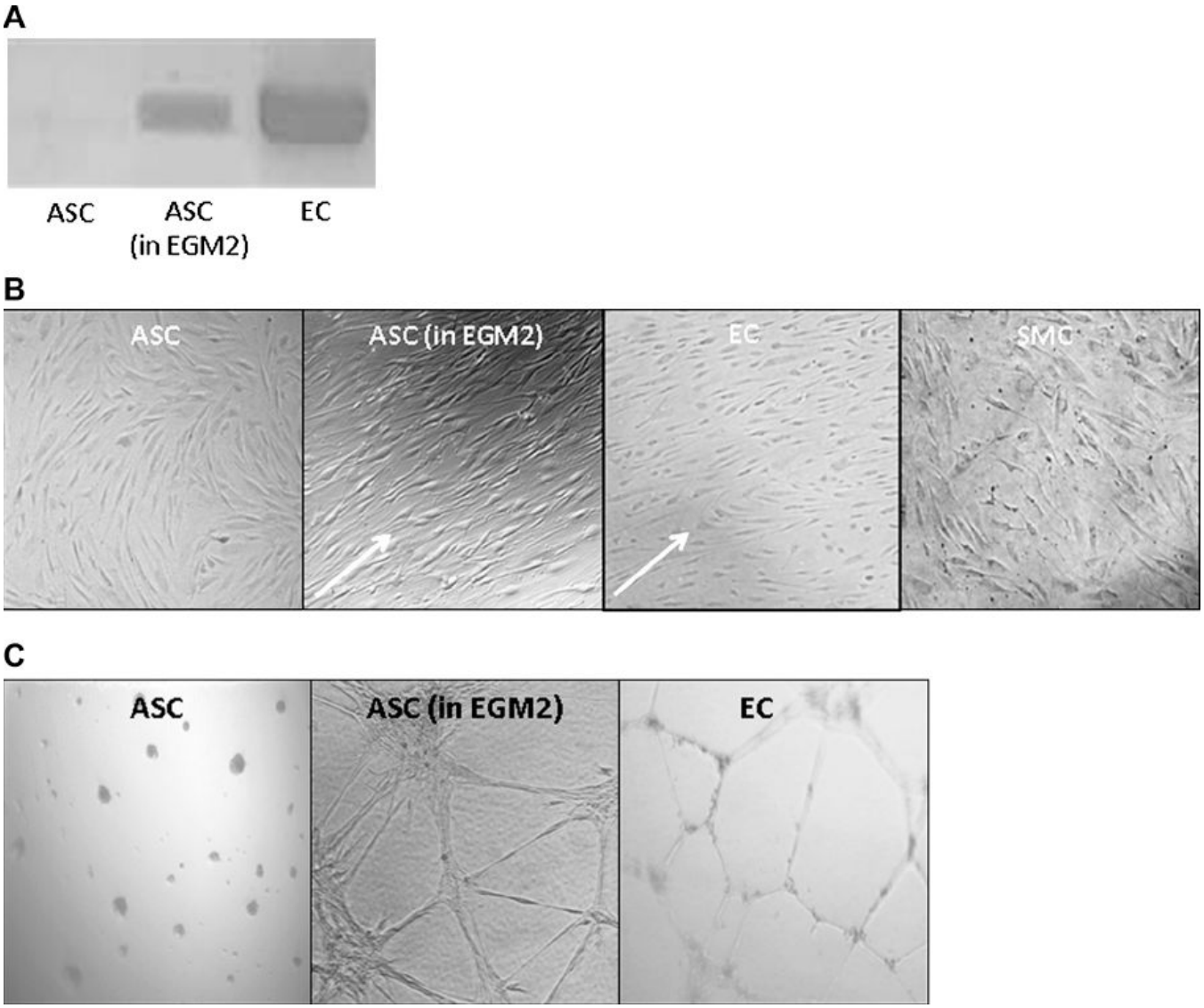

Figure 2. Acquisition of EC characteristics by ASC

Following culture in medium to induce endothelial differentiation (EGM-2 for two weeks), 15 randomly selected stem cell lines were evaluated for the acquisition of endothelial cell-specific traits: expression of CD31, morphologic alignment in the direction of shear stress, and cord formation upon plating on Matrigel. Representative examples of these experiments are shown: (A) RT-PCR for CD31, (B) phasecontrast micrograph (100x) of cells exposed to 12 dynes for 72h, and (C) phase-contrast micrograph (100x) of cells 24h after plating on Matrigel. ASC = adipose-derived stem cell; ASC (in EGM-2) = differentiated ASC; EC = endothelial cell (positive control); SMC = smooth muscle cell (negative control).

Table 1

Summary of patient characteristics.

Patient Characteristics (n=50)	
Age (yrs) ± std	59 ± 16
Age (yrs), n (%)	
<70	35 (70)
≥70	15 (30)
Gender, n (%)	
Male	32 (64)
Female	18 (36)
Body Mass Index, n (%)	
Normal or overweight (<30)	30 (60)
Obese (≥30)	20 (40)
Diabetes, n (%)	18 (36)
End-Stage Renal Disease, n (%)	8 (16)
Creatinine (mg/dl), mean ± std	2.0 ± 2.3
Peripheral Vascular Disease, n (%)	36 (72)
Indication for surgery:	
Arterial disease	36 (72)
Varicose veins	7 (14)
Hemodialysis access	4 (8)
Thoracic outlet syndrome	3 (6)