The Aesthetic Surgery Education and Research Foundation

Research

Adipose Stem Cell Function Maintained with Age: An Intra-Subject Study of Long-Term Cryopreserved Cells

Aesthetic Surgery Journal
2017, Vol 37(4) 454–463

DOI: 10.1093/asj/sjw197
www.aestheticsurgeryjournal.com
OXFORD UNIVERSITY PRESS

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Abstract

Background: The progressive decline in tissue mechanical strength that occurs with aging is hypothesized to be due to a loss of resident stem cell number and function. As such, there is concern regarding use of autologous adult stem cell therapy in older patients. To abrogate this, many patients elect to cryopreserve the adipose stromal-vascular fraction (SVF) of lipoaspirate, which contains resident adipose stem cells (ASC). However, it is not clear yet if there is any clinical benefit from banking cells at a younger age.

Objectives: We performed a comparative analysis of SVF composition and ASC function from cells obtained under GMP conditions from the same three patients with time gap of 7 to 12 years.

Methods: SVF, cryobanked under good manufacturing practice (GMP) conditions, was thawed and cell yield, viability, and cellular composition were assessed. In parallel, ASC proliferation and efficiency of tri-lineage differentiation were evaluated.

Results: The results showed no significant differences existed in cell yield and SVF subpopulation composition within the same patient between harvest procedures 7 to 12 years apart. Further, no change in proliferation rates of cultured ASCs was found, and expanded cells from all patients were capable of tri-lineage differentiation.

Conclusions: By harvesting fat from the same patient at two time points, we have shown that despite the natural human aging process, the prevalence and functional activity of ASCs in an adult mesenchymal stem cell, is highly preserved.

Level of Evidence: 5

Editorial Decision date: September 28, 2016; online publish-ahead-of-print November 5, 2016.

Mesenchymal stem cell (MSC) clinical trials listed on the US National Institutes of Health-based clinical trial registry surpassed 100 in 2015, indicating a great clinical interest in stem cell therapies for a multitude of currently devastating disorders. Adipose stem cells (ASCs) are mesenchymal, adult stem cells (MSCs) which reside in the

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stromal-vascular component of adipose tissue, with a primary function of tissue repair and expansion. Human ASCs have been shown to be a true stem cell population with clonal expansion capacity in vitro and the ability to differentiate toward cells of multiple tissue types including cartilage, bone, and muscle.[1] In addition, ASCs share many properties of MSCs from all tissue sources, including: plastic adherence in standard culture conditions, expression of nonspecific immunophenotype markers CD105, CD90, and CD73, and a lack of expression of CD34, CD45, CD14, CD11b, CD79a, CD19, and class-II major histocompatibility complex (MHC-II) molecules.[2] Like bone marrow-derived stem cells (BMSCs), ASCs are immunosuppressive and upon systemic injection, home to injured areas in response to chemical mediators of hypoxia, apoptosis, or inflammation.[3-6] Unlike BMSCs, ASCs can be easily obtained in a minimally invasive manner in clinically applicable numbers without the need of culture expansion and have a significantly greater angiogenic potential than BMSCs.[7,8] For these reasons, ASCs are an appealing stem cell population for cell-based therapies targeting tissue repair and regeneration.

As the field of cell therapy moves forward, particularly toward treating disorders affecting elderly populations, it will be critical to understand how donor demographics, such as age, impact stem cell efficacy. Clinical data on the impact of donor age on mobilized peripheral blood stem cells number has shown that in 400 subjects, donors over the age of 55 years have significantly lower frequency of CD34+ cells than younger donors.[9] Donor age also appears to affect bone marrow stem cell efficacy, with potency decreasing sequentially across populations of 18 to 30 years, 31 to 45 years, and more than 45 years[10] This data has been recapitulated in animal studies using MSCs to repair myocardial infarction in mice, showing older mouse and older human MSCs have significantly reduced regenerative capacity.[11-13] While it appears that there are intrinsic age related differences in purified stem cell populations, the impact of donor age on heterogeneous stromal cell populations, such as those extracted from adipose tissue as well as purified adipose stem cells, is not yet known.

The heterogenous, non-lipid laden population of adipose tissue has been termed stromal vascular fraction (SVF) and includes blood vessel-derived cells such as endothelial cells, smooth muscle cells, pericytes, and ASCs, as well as preadipocytes, hematopoietic-lineage cells, fibroblasts, and immune cells. Because of regulatory considerations for US clinical trials, SVF is often used in lieu of purified or culture expanded ASCs to achieve therapeutic effect. To isolate SVF, mechanically disrupted adipose tissue is enzymatically digested to release cellular components into a buffer solution, which is then centrifuged to pellet the dense stromal cell component. Currently, SVF is being used in 31 clinical trials for a multitude of therapeutic interventions including for autoimmune diseases, diabetes, gastrointestinal diseases, and chronic wounds.[14]

The purpose of this study was to evaluate intra-subject age effects in SVF and cultured adipose stem cells using cells that have been cryopreserved and long-term banked. To date, there are no studies that evaluated the biologic differences in SVF characteristics when harvested from the same person over extended periods of time. Studies examining the impact of age on ASC biology typically use subjects distributed into groups, usually with a small n value (6 subjects or fewer). Such comparisons across pooled groups is particularly difficult for adipose derived cells as significant differences in ASC properties within the same donor exist due to anatomical tissue region or harvesting procedures.[15] Therefore, subjects categorized only by age without standardizing all other variables may have significant protocol differences that make cross comparisons difficult.

In this study, SVF collected during past elective surgical procedures and banked under clinical conditions was compared with SVF collected in an identical manner from the same anatomic region after a secondary elective procedure seven or more years later. SVF compositions were compared based on analysis of progenitor and stem cell frequencies using flow cytometry quantification of established panels of cell surface markers. Differentiation potentials of ASC, to adipo-, osteo-, and chondrocyte lineages, were assessed and cell proliferative capacity across multiple passages was measured. Our results indicate that SVF subpopulation compositions are variable across donors but are consistent within the same subject despite aging. We did not observe any functional differences between stem cell of the first and second harvest from the same patient (young vs old).

METHODS

Human Subjects

The initial collection of adipose tissue and cell banking procedures were elective and occurred prior to study inception. This study was conducted from December 2013 to December 2015 in Pittsburgh, PA. Subjects who had independently banked cells as a subscription service and desired a second surgical procedure were eligible to participate in this study. De-identified adipose tissue was collected during elective body contouring procedures under approval by the Western Institutional Review Board for human research (Protocol #20122134). In total, three subjects were enrolled in the study between 2012 and 2014, including one female, aged 17 and 24 years at tissue collection (Subject 1, F17 and Subject 1, F24, respectively), and two males, one aged 21 and 29 years at tissue collection (Subject 2, M21 and Subject 2, M29), and the other aged

72 and 84 years at tissue collection (Subject 3, M72 and Subject 3, M84). The same surgeon (PBF) performed both primary and subsequent tissue harvesting procedures for all subjects using identical technique. The collected tissue was sterilely packaged in clinical shipping containers according to BioLife Cell Bank Dallas, LLC standard operating procedures.

Stromal Vascular Fraction (SVF) Isolation and Cryopreservation

Original cell isolations and banking were performed by Cytori Therapeutics, Inc, San Diego, CA. Detailed proprietary procedures were documented which included digestion enzyme type, its concentration, and cryo-preservation solution. Under confidentiality agreement, Cytori transferred isolation standard operating procedures to the University of Pittsburgh where secondary isolation procedures were replicated exactly for all subjects. Protocols were matched for all raw materials and controlled rate freezing procedures. SVF were banked in industry standard 25 mL bags designed for processing and freezing cord blood stem cells.

Flow Cytometry Analysis

Frozen cryobags with SVF were removed from liquid nitrogen storage and immediately placed it into a 37°C water bath. Cryopreservation solution was allowed to quickly thaw (< 5 minutes) until there was only a small amount of ice remaining. Cells were immediately diluted in media containing 10% fetal bovine serum (FBS) and centrifuged at 200 × *g* for 7 minutes to pellet. Cells were then prepared for analytical flow cytometry according to Zimmerlin et al.[16] Nine color, 14-parameter data files were acquired on a three-laser Gallios cytometer (Beckman Coulter) at a maximum of 10,000 events per second. Threshold was set on 4', 6-diamidino-2-Phenylindole, dihydrochloride (DAPI) fluorescence to exclude subcellular debris and up to 2.6 million events were acquired per sample. Offline compensation and analysis were performed using VenturiOne software (Applied Cytometry, Sheffield, UK). Mouse anti-human antibodies included: CD105-FITC (Fitzgerald, #61R-CD105-DHUFT), CD73-PE (BD Biosciences, #550257), CD146-biotin (Miltenyi Biotec, #130-092-852), CD14-PE-Cy5 (Beckman Coulter, #IM2640U), CD31-PE-Cy7 (Biolegend, #303117), CD90-APC (BD Biosciences, #559869), CD34 APC-Alexa 700 (Beckman Coulter, #A86354).

SVF Viability and Proliferation

Frozen SVF was independently thawed in parallel at Indiana University (IU) and the University of Pittsburgh (Pitt) as described above and pre-warmed complete growth medium (EGM-2MV, Lonza #CC-3202) was used to dilute cryopreservation solution ten-fold (1 part cryo solution: 9 parts growth media). Cell viability was measured using trypan blue exclusion and a Countess automated cell counter (Invitrogen, #C10227) (Pitt) or with a hemocytometer (IU). At both facilities, SVF was plated in techno plastic products (TPP) Tissue Culture T75 Flasks (MidSci #TP90076) at 50,000 cells/cm^2 in 10 mL culture media with standard growing conditions (37°C, 5% CO2). Cells were allowed to attach overnight, no more than 18 hours, after which unattached cells were washed with Dulbecco's PBS (without Ca++ and Mg++). Plated cells were lifted with 0.25% Trypsin, no ethylenediaminetetraacetic acid (EDTA) (Fisher, #MT-25-050-CI), counted and re-plated at 5000 cells/cm^2 in triplicates in 6-well plates, 2 mL of media per well. Cells were continuously expanded until passage 8, lifting at 75% to 80% confluency. Cell population doubling rates (PDR) were calculated for both IU and Pitt and compared for consistency and both data sets were included in the analysis.

Tri-Lineage Differentiation

At passage 3, adherent cells were evaluated for differentiation capacity to adipo-, osteo-, and chondrocytes using commercially available StemPro media.

Adipogenesis

To assess adipogenic potential of cultured cells were lifted, counted and plated at 60,000 cells/cm2 in triplicate in both 12-well and 48-well plates. Cells were allowed to adhere overnight and then culture media was removed and replaced with StemPro adipogenesis differentiation media (Invitrogen #A10070-01) for 14 days, changing every 2 to 3 days. Control cells were identically cultured with basal media that did not contain adipogenic supplements, also in triplicate. To quantify lipid inclusions, fixed cells in 48-well plates were treated with 10 µg/mL Nile Red (Molecular Probes #N1142) for 30 minutes after with the fluorescent intensity was measured on a Tecan M200Pro plate reader and normalized to DAPI.

Osteogenesis

Cells were plated in 24-well plates with Nunc* Thermanox* Coverslips (Fisher Scientific #12-565-88) at 50,000 cells/cm2 in triplicate. After cells attached overnight, media was replaced with StemPro osteogenesis differentiation media for 21 days, changing every 2 to 3 days. To confirm osteogenesis, cells were stained using Alizarin Red S (ARS) stain solution, prepared with enhanced calcium specificity at a pH of 4.1 to 4.3 as recommended, using ammonium hydroxide. At the end of differentiation step, cells in 24 well-plates were fixed with 4% paraformaldehyde, washed with excess distilled water, stained with Mayer's haematoxylin for 2 to 5 minutes, rinsed with water, and

then stained with 40 mM ARS for 20 min with gentle shaking. The unincorporated dye was washed from the cells four times with excess distilled water while shaking for 5 minutes each wash and cells were cover-slipped with Permount mounting media. Due to temporal changes and reduced osteogenic gene expression at later time points, quantitative reverse transcription–polymerase chain reaction was performed on cells plated in triplicate in 12-well plates at 3000 cells/cm2 and differentiated for 5 days.

Chondrogenesis

Suspended cells at 5 × 10^5/mL were aliquoted into 10, 15-mL polypropylene conical tubes, with 0.5 mL per tube. Cells were centrifuge at 300 × *g* at 21 °C for 5 min to form a pellet. The tops of the conical tubes remained loose for gas exchange and samples were incubated overnight. The following day, media was replaced with either incomplete differentiation media containing DMEM (Invitrogen # 11054-020), 2 mM L-glutamine (Invitrogen #25030-081), 1X Insulin-Transferrin-Selenium-Plus (BD Biosciences # 354352), 40 µg/mL L-proline (Sigma cat# P5607), 0.1 µM Dexamethasone (Sigma Aldrich, #D4902), and 5µg/mL gentamicin (Invitrogen, #15710-064) or complete differentiation media comprised of incomplete media supplemented with 50 µM L-ascorbic acid 2-phosphate (Invitrogen, #A8960) and 10 ng/mL human transforming growth factor (TGF) beta (R&D Systems, 243-B3-002). Media was replaced every 2 to 3 days for 28 days. During each exchange, pellets were gently agitated to ensure that they would not adhere to the tube wall. At the completion of cell differentiation, two control and chondrocyte pellets were snap frozen in optimal cutting temperature gel, cryosectioned at 5 µm, and stained with Safranin Orange to visualize proteoglycans.

To quantify gene adipo- and osteo- gene expression, ribonucleic acid (RNA) was extracted using an RNeasy mini kit (Qiagen #74104) with DNAse cleanup. Due to the durability and negative charge of cartilage matrix, RNA was extracted from chondrogenic pellets using Trizol extraction and Kimble BioMasherII Closed System Micro Tissue Homogenizer (Fisher, # K7496250010) after which RNA was purified with the RNeasy mini kit as described. cDNA synthesis was performed using 500 ng RNA in 10 µL RNAse free water, random primers (Invitrogen, # 48190011), M-MLVRT (Invitrogen, #28025-013) and appropriate buffers. Relative gene expression for each study subject was determined using the ΔCt method with experimental gene expression compared to glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

qPCR Primers

Genes of interest included peroxisome proliferator-activated receptor gamma (PPAR-γ or PPARG) (Life Technologies, #4331182 Hs01115513_m1), fatty acid binding protein 4 (FABP4) (Life Technologies, #4331182 Hs01086177_m1), alkaline phosphatase (ALP) (Life Technologies, 4331182 Hs01029144_m1), and cartilage oligomeric matrix protein (COMP) (Life Technologies, #4331182 Hs01561085_g1). GAPDH (Life Technologies, #4331182 Hs02758991_g1) was used as a housekeeping primer for all samples, having been shown as consistently expressed before and after differentiation (data not shown).

Data Analysis and Statistical Software

For all groups, data is expressed as mean +/- standard deviation. For multiple comparisons, an analysis of variance was performed with Tukey-Kramer adjustment using SAS9.4 statistical software. Significant differences were determined using $\alpha = 0.05$.

RESULTS

ASC Frequency in SVF is Not Decreased Due to Donor Age

SVF was isolated from adipose tissue collected in two consecutive elective cosmetic procedures, separated by 7 or more years, and cryopreserved under clinical conditions for three subjects, one female (Subject 1: 17 years and 24 years of age) and two males (Subject 2: 21 years and 29 years and Subject 3: 72 years and 84 years of age). After thaw, all nucleated cells were enumerated and evaluated by analytical flow cytometry based on previously established cell surface signatures and gating strategy.

Analytical flow cytometry results for SVF subpopulations are shown in Table 1. Data suggests that Subject 3, the oldest study participant, had significantly reduced percentages of non-hematopoietic, CD45- cells, with 14.13% and 15.49% at age 72 and 84 years (respectively) compared with Subject 1, 28.98% (17 years) and 32.02% (24 years) and subject 2, 42.43% (21 years) and 36.30% (29 years). Subject 3 also had significantly reduced early CD31 + endothelial cell populations, including endothelial progenitors (CD45-CD31 + CD34 + CD146-) and transitional endothelial (CD45-CD31 + CD34 + CD90 + CD146 +). However, there were no consistent differences between subjects for mature endothelial cells (CD45-CD31 + CD34-, data not shown). Unexpectedly, Subject 3 had the highest frequency of ASCs (CD45-CD31-CD34 + CD90 + CD105 + CD73 +) and endothelial related CD45 + CD34 + progenitors, with consistently higher cell counts at both 72 and 84 years of age.

Older Adipose Cells have Equal Population Doubling

Population doubling rate for all subjects was approximately 0.7 days (16.8 hours) and was not significantly different

Table 1. Quantitative Statistics on SVF Yield and Analysis of Flow Cytometry Results for Study Subjects

		Non-hematopoietic	Endothelial progenitors	Lymphatic endothelial	Adipose stem cell	Preadipocytes
	SVF / mL Lipo (10^3)	CD45-	CD45-CD31+ CD34+CD146-	CD45-CD31+ CD34+ CD90+ CD146+	CD45-CD31- CD34+CD90+ CD73+CD105+	CD31-CD146- CD34 + 90+
Subject 1, F17	313	28.98	2.63	10.40	1.21	4.28
Subject 1, F24	243	32.03	3.54	12.22	1.05	11.00
Subject 2, M21	158	42.43	5.64	9.35	0.76	11.06
Subject 2, M29	145	36.30	6.26	8.65	1.28	11.94
Subject 3, M72	188	14.13	0.92	2.13	2.27	9.86
Subject 3, M84	165	15.49	1.49	3.36	2.27	2.17

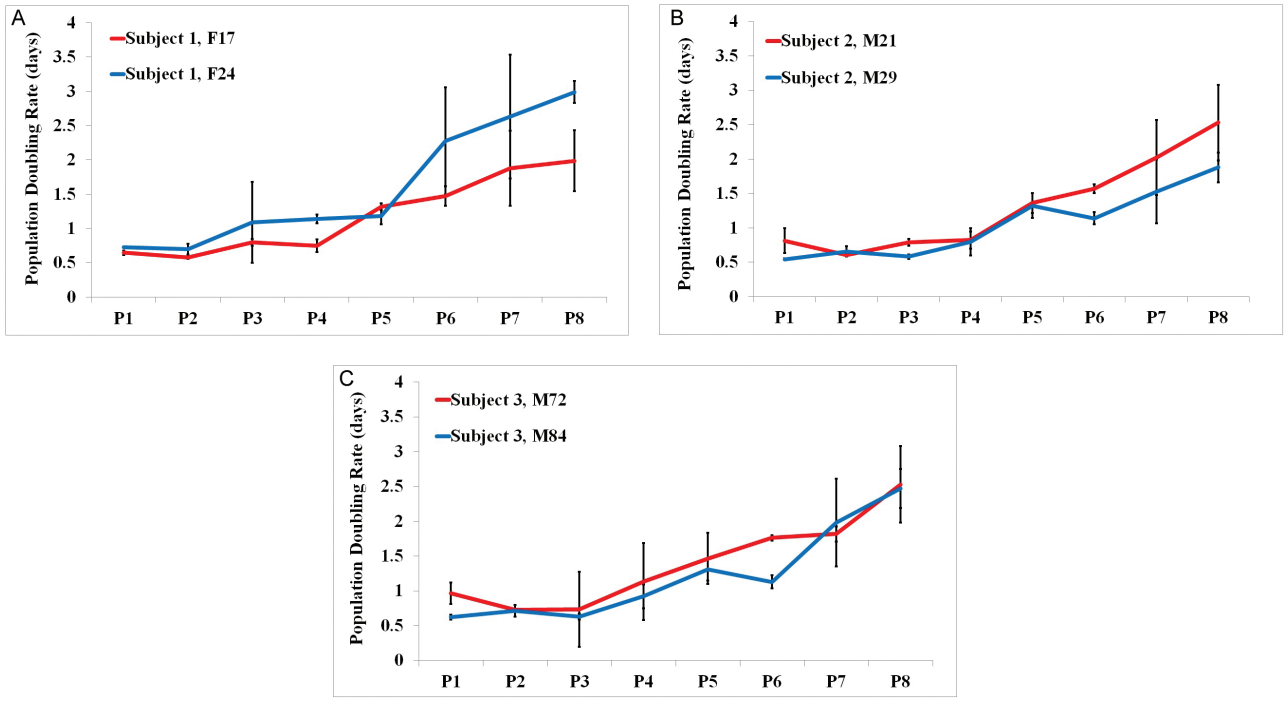

Figure 1. Population doubling (PD) rate for study subjects comparing cell proliferation after donor aging. Subject 1 (A), subject 2 (B) and subject 3 (C). Data presented as mean ± standard deviation. N = 2 studies performed independently by Pitt and IU, with n = 3 replicates for each study.

until passage 5 (Figure 1). For all samples, cell-doubling rate slowed after the fifth passage and with each consecutive passage until passage 8. There was no significant difference between young and old cell doubling rate for any passage number.

Adipose Cell Tri-differentiation

Lipid Accumulation and Adipogenic Gene Expression

To assess cell adipogenic capacity, cells were plated at confluency and allowed to attach overnight before replacing standard culture media with commercially available adipogenic media. Cells in basal media without supplemental growth factors served as negative controls. All samples in inductive media had intracellular lipid accumulations by day 3, which increased in number and size until day 14 (Figures 2A-C).

After cell fixation, Nile Red was used to quantify lipids and the fluorescent intensity was normalized to cell number, as measured with DAPI. Our results indicate that ASCs from Subject 2, (Male, 21 and 29 year old [y.o.]) had significantly lower lipid content per cell than Subject 1 (Female, 17 and 24 y.o.) or Subject 3 (Male, 72 and 84 y.o.). While Subject 1 had consistent lipid accumulation at age 17 and

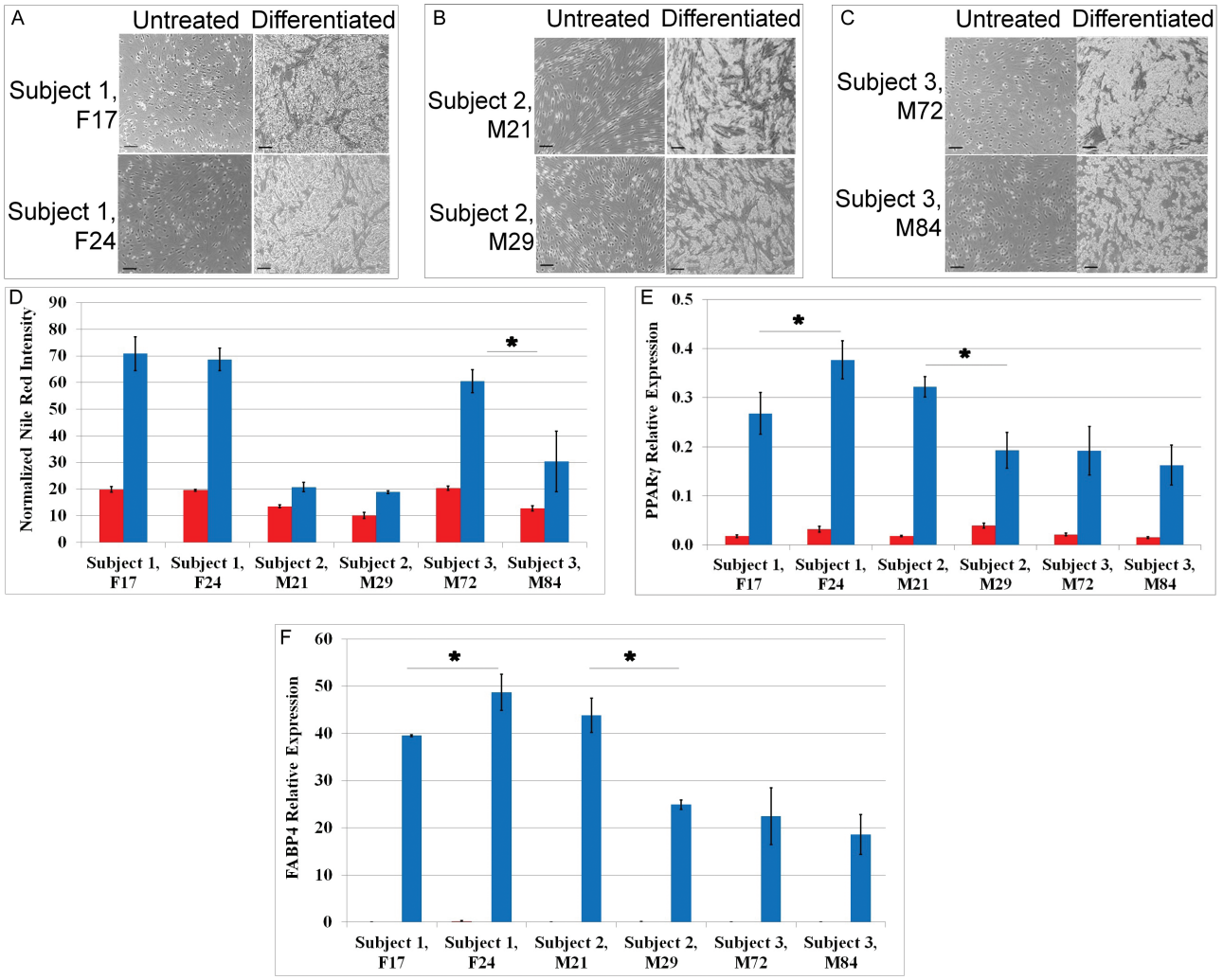

Figure 2. (A-C) Representative micrographs of control and adipocyte differentiated passage 3 cells, n = 3 replicates per group. Scale bars are 100 μM. (D) Nile Red quantification of lipid accumulation in control (red bars) and treatment (blue bars) groups, n = 4 per group. (E, F) Relative expression of early (PPARγ) and mature (FABP4) adipocyte gene expression in control (red bars) and differentiation treatment (blue bars) groups, n = 3 replicates per group. Asterisks indicate $P < 0.05$.

24 years, Subject 3 showed significantly decreased lipid accumulation at age 84 compared with 72 years of age (Figure 2D).

A comparison of cell expression of early (PPARγ) and mature (FABP4) adipocyte genes indicated that ASCs from all subjects underwent adipogenesis as PPARγ and FABP4 expression was increased in differentiation treated cells compared with corresponding controls ($P < 0.05$) (Figure 2E). Comparing old and young cells, there was no consistent trend across all three patients. While Subject 1 had significantly increased adipogenic gene expression in older cells (24 vs 17 y.o.), Subject 2 adipogenic gene expression was decreased in older cells (29 vs 21 y.o.). No change in adipogenic gene expression was observed in the oldest subject (72 and 84 y.o.).

Osteogenesis and Calcium Formation

ASCs from all subjects underwent a morphology change when treated with osteogenic media and had significantly increased expression of ALP at 5 days. Alizarin red staining of controls and experimental cultures showed significant calcium accumulation by day 21 (Figures 3A-C). Intra-subject differences in alkaline phosphatase expression were only observed in Subject 2, with significantly higher expression in cells harvested at 29 years compared to cells extracted at 21 years ($P < 0.05$) (Figure 3D).

Chondrogenesis

Safranin-O staining confirmed that all subject ASCs underwent chondrogenic differentiation and produced proteoglycans by day 28 (Figures 4A-C). Chondrogenesis

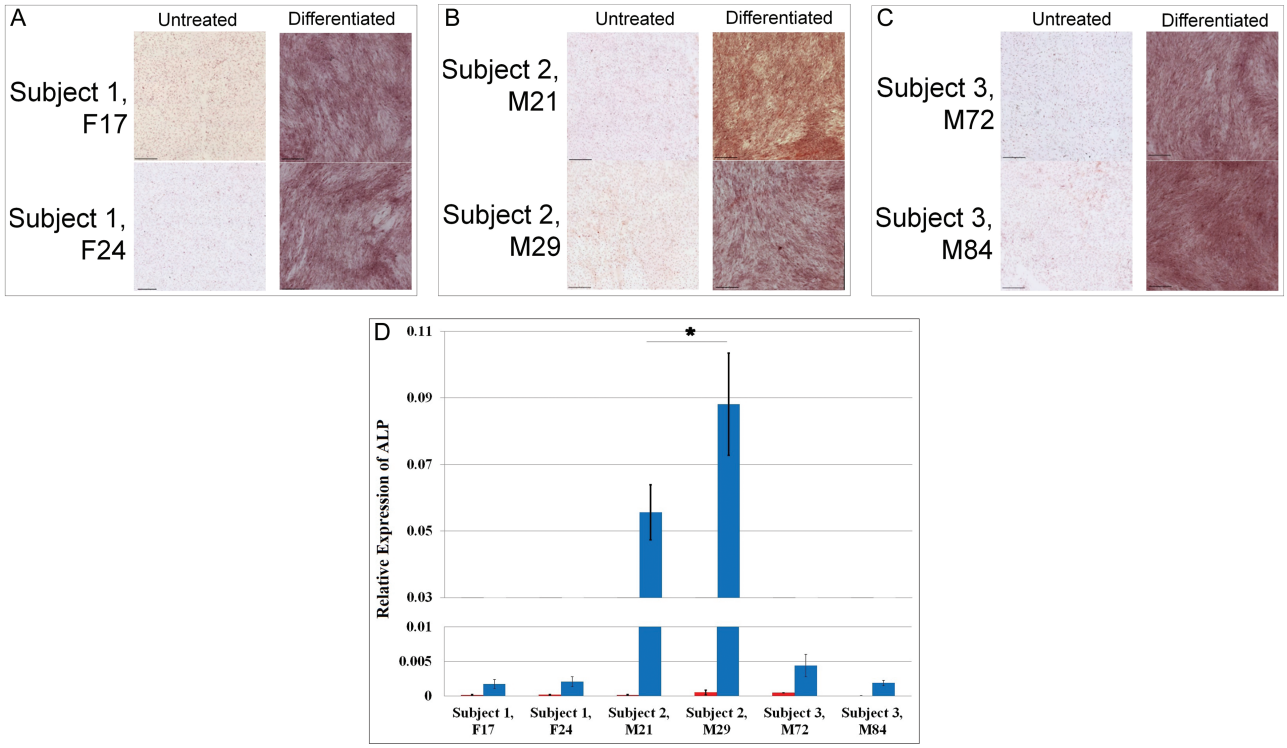

Figure 3. (A-C) Representative micrographs of control and osteocyte differentiated passage 3 cells stained with Alizarin red, n = 3 per group. Scale bar is 100 μM. (D) Relative expression of alkaline phosphatase gene expression in control (red bars) and differentiation treatment (blue bars) groups, n = 3 replicates per group. Asterisks indicate $P < 0.05$.

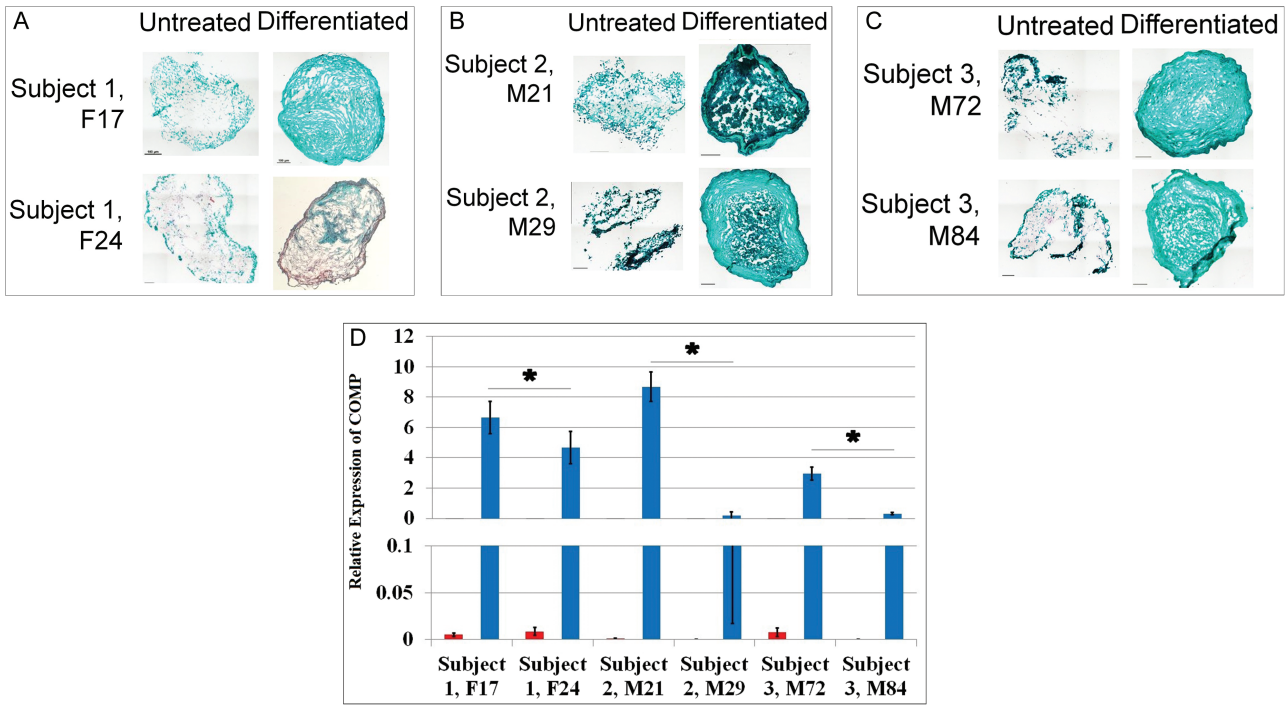

Figure 4. (A-C) Representative micrographs of control and chondrocyte differentiated passage 3 cells stained with Safranin-O, n = 2 replicates per group. Scale bar is 100 μM. (D) Relative expression of cartilage oligomeric matrix protein gene expression in control (red bars) and differentiation treatment (blue bars) groups, n = 3 replicates per group. Asterisks indicate $P < 0.05$.

was confirmed for all cells by increased expression of COMP, which was consistently had a decreased expression in old vs young cells for all study subjects ($P < 0.05$) (Figure 4D).

DISCUSSION

Adipose tissue is a readily available source of adult mesenchymal stem cells. For most patients, clinically relevant numbers of stromal vascular cells can be obtained from lipoaspirate tissue after minimally invasive liposuction procedures. Therefore, stromal vascular cells, which contain adipose stem cells, are an important cell therapy modality and are already being used in clinical trials for a variety of targets.[17]

Previous literature has suggested that there are no significant decreases in adult stem cell viability or function with long-term cryopreservation.[18-25] Studies conducted with adipose derived stem cells have also shown no detrimental effects due to cryopreservation.[26,27] Using passage 1 ASCs cryopreserved for at least one month, Goh et al have reported that cryopreservation had no significant effect on ASC proliferation rate in culture or the ability to differentiate into mature adipocytes. However, the authors have noted that the frequencies of colony forming units (CFU) for osteogenesis slightly diminished after freezing step. Additional work by Gonda et al confirmed that the proliferative capacity and multipotency of human ASCs before and after long-term (6 months) cryopreservation have maintained. However, while our study is the first to show that ASC function is maintained for ten or more years, because of the unique subject set obtained, we were not able to establish a baseline for cell functionality before cryopreservation. Therefore, it is possible that in fact a decrease in cell function with age does occur, but is equal to the decrease in cell function with long-term cryopreservation. This is a significant limitation of this study.

Because ASCs can be stored for extended periods without loss of function, the goal of this study was to determine if it would be advantageous for younger patients to bank cells for future clinical use. Literature on the impact of donor age on stem cell function is conflicting, reporting both no significant or detrimental effects. Mojallal et al isolated ASCs from 42 women and compared cell growth rates for those aged ≤40 or above 40 and concluded that there was no significant difference between the cell yield and proliferative capacity between the groups.[28] In contrast, Kornicka et al, reported that cells from younger donors (>20 years of age) had the fastest population doubling in comparison to patients 50 to 70 years of age, which had a 2.7-fold longer PD time.[29] Our results indicate that there was no significant change in population doubling rate for ASCs after donor aging. For all study subjects, PDT was equivalent between passages 0 to 4 and slowed with sequential passaging at passage 5 and beyond. These findings are similar to those reported by Wall et al, in which cultured ASC cell doubling rate began to slow at passage 5 and was significantly different at passage 6 and beyond.[30]

Comparison of PDT between the patients suggested no significant differences existed for any subject despite large differences in age (17, 21, 24, 29, 72, and 84). These measurements were made in parallel in two laboratories, at Indiana University and University of Pittsburgh and produced similar results. Collectively, our data confirms published studies that showed ASC yield and their hardiness are not correlated with donor age.[28,31,32] Such results seem counterintuitive when considering alternative literature suggesting that bone marrow stem cell (BMSC) properties declines with age and BMSCs have reduced contribution to tissue regeneration.[33,34] One possible explanation, and limitation of our findings, could be that quantifying cell doubling rate does not independently describe cell hardiness and overall functionality. For example, Alt et al measured cell proliferation and performed an expression analysis for markers of self-renewal and differentiation capacity for groups of patients including 30 to 40 year old ($n = 17$; mean age 34.4 ± 1.6) and greater than 50 years ($n = 8$; mean age 61.33 ± 7.4). While PDT was not significantly different between these two groups, genes related to senescence such as CHEK1 and cyclin-dependent kinase inhibitor, $p16^{ink4a}$, were significantly increased, telomerase activity was reduced, and cells from older patients had a significant upregulation of NFκB, TNFα, and the genes for their corresponding receptors.[35] Therefore, additional analysis on cell senescence, autophagy, and deoxyribonucleic acid (DNA) mutations may yield a more complete picture of cell hardiness.

Unexpectedly, analytical flow cytometry results of SVF subpopulations suggested that ASC frequency in the stromal vascular fraction is highly patient specific and did not decrease with donor age. Such results contradict studies on aging population differences for bone marrow-derived MSC frequency[36] and circulating endothelial progenitor cells.[37] While our oldest subject was 84 at the time of study participation, there was no observed decrease in SVF yield or ASC proportion of SVF compared with the other two subjects, aged 24 and 29. We further showed that passage 3 ASCs from all patients underwent robust differentiation to three cell types, adipocytes, osteocytes, and chondrocytes, indicating stem cell function was maintained. These results are similar to those found in aged animal studies which explored adipogenic and osteogenic potential of ASCs harvested from senescence-accelerated osteoporotic mice.[38] Comparing ASCs from control and aged mice showed no evidence of telomere shortening, increased cellular senescence, or impairment in either the early or late

phases of osteogenic differentiation. These study results along with our findings support the use of ASCs for cell therapies for age-associated diseases such as osteoporosis.

It is known that pluripotent multi-lineage differentiating stress enduring (MUSE) cells reside within adipose tissue and have low proliferative and telomerase activity, activated primarily through cellular stressors such as low oxygen or high pH.[39] Due to the low innate activity of MUSE cells, the population may be maintained with age in a quiescent state within the cellular niche. Such cells are identified as a component of SVF using flow cytometry for positive selection for both the human pluripotency marker stage-specific embryonic antigen 3 (SSEA3) and CD105, a mesenchymal marker, with flow cytometry.[40] Upon activation, MUSE cells may be induced to differentiation outside of the typical mesodermal lineages, including toward hepatocyte and neuronal cell types and may therefore have additional applications in regenerative medicine applications that have not yet been investigated. MUSE cells also have increased expression of CXCL2, a critical chemokine involved in stem cell homing, which may improve the homing capacity of systemically injected cells to areas of tissue injury or stress.[41] Future studies should include identification of this subpopulation within SVF in addition to traditional ASC populations.

Another limitation of this study includes the small sample size of study subjects. While we consider our data set of genetically identical patients to be a novel and compelling method for evaluating age effects on stem cell function, there were significant challenges in obtaining patients who met the study criteria of having stem cells banked long term under GMP conditions. As such, we consider our study findings preliminary and will continue to seek out eligible study participants.

CONCLUSION

Results on these study confirm previous reports by Wu et al that the clinical applicability of adipose-derived mesenchymal stem cells is conserved despite age.[42] These results are highly relevant for ASC-based therapies considering the number of applicable clinical targets for stem cell transplantation which occur in elderly patients. Our results suggest that significant differences in SVF subpopulations and ASC function are patient specific and do not appear to change much after aging 7 to 12 years.

Acknowledgements

The authors gratefully acknowledge the University of Pittsburgh Center for Biologic Imaging as provider of instrumentation

Disclosures

The authors declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

Funding

This study was funded by a grant from the Aesthetic Surgery Education and Research Foundation (ASERF). Funding was used for laboratory supplies (including experimental materials and laboratory costs) and laboratory technician's salary.

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