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Influence of Donor Age and Comorbidities on Transduced Human Adipose-Derived Stem Cell *In Vitro* Osteogenic Potential

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Abstract

Human adipose-derived mesenchymal stem cells (ASCs) transduced with a lentiviral vector system to express bone morphogenetic protein 2 (LV-BMP-2) have been shown to reliably heal bone defects in animal models. However, the influence of donor characteristics such as age, sex, race, and medical co-morbidities on ASC yield, growth and bone regenerative capacity, while critical to the successful clinical translation of stem cell-based therapies, are not well understood. Human ASCs isolated from the infrapatellar fat pads in 122 ASC donors were evaluated for cell growth characteristics; 44 underwent additional analyses to evaluate *in vitro* osteogenic potential, with and without LV-BMP-2 transduction. We found that while female donors demonstrated significantly higher cell yield and ASC growth rates, age, race, and the presence of co-morbid conditions were not associated with differences in proliferation. Donor demographics or the presence of comorbidities were not associated with differences in in vitro osteogenic potential or stem cell differentiation, except that transduced ASCs from healthy donors produced more BMP-2 at day 2. Overall, donor age, sex, race, and the presence of co-morbid conditions had a limited influence on cell yield, proliferation, self-renewal capacity, and osteogenic potential

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AUTHOR CONTRIBUTIONS

KC and JAB were responsible for designing the study, data acquisition, interpreting results, and drafting manuscript.
MCG was responsible for statistical analyses, data acquisition, and drafting manuscript.
SWC was responsible for data acquisition and drafting manuscript.
SB was responsible for performing the statistical analysis and drafting manuscript.
OS was responsible for designing the study and data acquisition and drafting manuscript.
JT and DE were responsible for performing and analyzing flow cytometry data and revising manuscript.
NH, DAO, and DBL were responsible for harvesting specimen and revising manuscript.
RH and DBK were responsible for designing the study methodology and revising manuscript.
JRL was responsible for designing the study methodology, interpreting results, drafting and revising manuscript.

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for non-transduced and transduced (LV-BMP-2) ASCs. These results suggest that ASCs are a promising resource for both autologous and allogeneic cell-based gene therapy applications.

INTRODUCTION

Bone loss is a common yet challenging clinical problem that may be seen in the setting of fracture nonunion, revision total joint arthroplasty, and spine pseudoarthrosis.(1) Despite advances in orthopaedic care, a consistently satisfactory solution for large bone defects remains elusive. Four critical elements are required for bone healing: osteogenic cells, osteoinductive growth factors, an osteoconductive scaffold for new bone formation, and adequate blood supply.(2) Autograft tissue is the current gold standard graft material as it provides the first three elements.(3) However, it is limited in quantity and can be associated with significant harvest site morbidity.(4) Therefore, there is considerable interest in developing alternative bone repair strategies.

Tissue engineering strategies that incorporate mesenchymal stem cells (MSCs) are a promising approach for bone regeneration.(1) Researchers have long investigated MSCs for their ability to induce bone formation and healing.(5–8) However, MSCs alone may not induce an adequate biological response in the more stringent biological environments often encountered in clinical cases of bone loss.(9, 10) In such cases, MSCs combined with an osteoinductive growth factor may be needed to mount an adequate healing response. *Ex vivo* regional gene therapy using a lentiviral vector containing the DNA for bone morphogenetic protein 2 (BMP-2) is thus an attractive tissue engineering strategy as it allows for the delivery of osteoprogenitor cells (i.e. MSCs), and a sustained osteoinductive signal (BMP-2) to a specific anatomic site to promote bone repair.(1, 11) Prior studies have demonstrated the potential of *ex vivo* regional gene therapy in clinically relevant animal models of bone healing.(1, 12–15)

An important step for the clinical translation of *ex vivo* regional gene therapy for bone repair, as well as other cell-based cell therapies, is the identification of an optimal MSC source. MSCs were first isolated from bone marrow and have historically received the most attention from investigators.(16) However, BM-MSC harvest is a painful and invasive procedure and there are limited numbers of stem cells in bone marrow aspirates, thus prompting a search for alternative cell sources. MSCs have been isolated from other tissues including adipose, skeletal muscle, periosteum, and skin, many of which have been successfully used in gene therapy approaches for bone repair in preclinical models. (1, 17–19) Adipose-derived mesenchymal stem cells (ASCs) have emerged as a particularly promising MSC source. Compared to BM-MSCs, ASCs demonstrate several advantages including: a less invasive acquisition from a variety of anatomical areas; a higher yield of MSCs; increased proliferation rate *in vitro*; and comparable if not superior osteogenic properties.(20–22) These clinical advantages have prompted interest in the development of an *ex vivo* regional gene therapy application utilizing transduced ASCs.(13, 23)

A remaining question for the successful clinical adaptation of ASC-based regional gene therapy is the influence of donor age and co-morbid conditions on ASC abundance, proliferation, and osteogenic potential. This is particularly important in the setting of

autologous cell therapy, where patients will rely solely on the regenerative capability of their own tissue. The effect of age on ASC function has been studied for both non-human and human cells, but discrepancies exist between studies.(24–30) Furthermore, it is unclear whether adipose tissue harvested from elderly patients with various inflammatory, metabolic, or immunosuppressed conditions (a demographic which has the potential to comprise many patients in bone loss scenarios in orthopaedics) will have adequate ASC function and bone regenerative potential. Several studies have demonstrated differences in ASC yield, proliferation, and differentiation potential that correlate with donor characteristics such as BMI, and comorbidities including diabetes, cardiovascular disease, and osteoporosis. However, the current literature is conflicting and typically involves evaluation of ASCs from only a small number of donors.(29, 31–36) In addition, at present there are no studies evaluating the aforementioned parameters in *transduced* ASCs for gene therapy applications.

The purpose of this study was to evaluate the effects of age, sex, race, and co-morbid medical conditions on ASC yield, proliferation, and *in vitro* osteogenic potential in both transduced and non-transduced cells, in order to better characterize the full clinical potential of human ASCs for cell-based gene therapy applications for bone repair. We hypothesized that increasing age and the presence of medical comorbidities would negatively affect cell yield and proliferation, as well as osteogenic potential of transduced ASCs.

MATERIALS AND METHODS

Human Cell Isolation and Culture

Institutional Review Board approval was obtained prior to collection of human tissue. Human adipose tissue was collected in a sterile fashion from the infrapatellar fat pads of patients undergoing primary total knee arthroplasty (TKA) for osteoarthritis at our institution. The fat pad is partially excised and discarded in a typical TKA. Age, sex, race/ethnicity, and medical comorbidities were recorded for each de-identified sample. Co-morbid patients were defined as those with medically treated metabolic or inflammatory diseases including diabetes mellitus, rheumatoid arthritis, or coronary artery disease. Patients receiving immunosuppressive medications and active smokers were also considered co-morbid. Exclusion criteria included patients greater than 80 years old, congenital disorders of bone and cartilage, osteonecrosis, active infection, a history of infection in the operative knee, and revision TKA.

The collected adipose tissue was processed using previously published protocols to obtain the stromal vascular fraction (SVF).(13, 37) Briefly, adipose samples were extensively washed with Dulbecco's PBS (DPBS, Lonza, Basel, CH) to remove debris and red blood cells. The tissue was mechanically digested with scissors and then enzymatically digested with 0.1 % collagenase (Sigma-Aldrich, St. Louis, MO) at 37 °C for 90 minutes. The solution was filtered and then washed three times with DPBS. The resulting SVF was resuspended with Dulbecco's modified eagle medium (DMEM; Corning, Corning, NY) containing 10 % fetal bovine serum (Gibco, Amarillo, TX) and an antibiotic mix containing 100 unit/mL penicillin, 100 μg/mL streptomycin and 250 ng/mL Amphotericin B (Lonza, Basel, CH).

Isolated SVF cells were counted with a TC20™ automated cell counter (BioRad, Hercules, CA) using trypan blue and then plated for expansion at a concentration of 2–3 × 10^6 cells per 10 cm dish. Cells were maintained at 37 °C and 5 % CO_2. Culture medium was replaced every 3–4 days. Cells were passaged weekly; adherent cells were trypsinized and replated at a density of 0.7 – 1.0 × 10^6 cells per 10 cm dish.

All donors were analyzed for initial cell yield and growth characteristics as determined by cell numbers at each successive passage. At passage 3, ASCs from a subset of 44 donors (21 healthy, 23 co-morbid) underwent immunophenotypic analysis, transduction with a lentiviral vector system to express BMP-2, and subsequent *in vitro* analysis for osteogenic potential as detailed below. These 44 donors reached a threshold number of cells by passage 3 which was required in order to perform all *in vitro* analyses.

Immunophenotypic Characterization

ASC cell-surface marker expression was characterized using multicolor flow cytometric (FACS) analysis. The CD marker panel was based on the criteria described by the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT).(38)

After trypsinization, cells were washed and then re-suspended in BD Pharmingen Stain Buffer (BD Biosciences, Franklin Lakes, NJ). Aliquots of the suspensions (150 μl) were used for cell counting with trypan blue. After counting, the remaining solution was then re-suspended at a concentration of 5 × 10^6 cells/mL. Aliquots of the suspension (100 μl) were then transferred to 1.5 mL eppendorf tube and stained with the following antibodies: CD44, CD73, CD90 and a hMSC negative cocktail (CD34, CD11b, CD19, HLA-DR). Unstained and single-color controls were also prepared. The tubes were incubated on ice in the dark for 30 minutes. The cells were then washed twice and resuspended with 200 μl of Pharmingen Stain Buffer prepared with DAPI (working concentration of 1 μg/mL). Analysis was performed using a BD FACSAria III (BD Biosciences). Compensation was set up with single color controls and unstained cells. Forward-scatter and side-scatter were used to exclude debris and doublets. Data were analyzed using FlowJo software (FlowJo LLC, Ashland, OR).

Lentiviral Transduction

ASCs were transduced with a two-step transcriptional amplification (TSTA) vector system, as described in previously published protocols.(39–41) Briefly, the TSTA system uses two lentiviral (LV) vectors: the GAL4-VP16 transactivator vector (LV-RhMLV-GAL4-VP16) and the transgene expression vector encoding BMP-2 (LV-G5-BMP-2).

Lentiviral vectors were generated by transfecting 293T cells (American Type Culture Collection, Manassas, VA) as described in a previous protocol.(40, 42) To determine functional titer of lentiviral vectors, HT-29 cells (ATCC, Manassas, VA) were transduced with diluted vector prep, then genomic DNA was extracted at three days after transduction. Lentiviral vector copy and human cell number in genomic DNA were quantified by QX200 droplet digital PCR (ddPCR) system (BioRad, Herclus, CA) using HIV-1 psi region and

human Syndecan-4 (SDC-4) primers and probes. LV titer was calculated from the vector copy number per cell.

After reaching passage 3, ASCs were plated at a density of 1×10^6 cells/dish. The next day, cells were transduced with the viral vectors at a multiplicity of infection of 3/3 in the presence of 8 μg/ml of polybrene. The cells were incubated overnight and the next day the medium was aspirated and replaced to remove extracellular virus.

In Vitro BMP-2 Production

BMP-2 production of transduced cells was evaluated with two 10 cm dishes of 1×10^6 transduced cells. After overnight transduction, as described above, cells were washed and resuspended in fresh media. Cells were maintained in culture with regular media changes. BMP-2 production over a 24 h period was assessed from collected supernatant at 2-, 7-, and 14-days post-transduction. BMP-2 was quantified by a commercial BMP-2 ELISA kit (Quantikine, R&D systems, Minneapolis, MN). Samples were run in duplicate and averaged across the two plates used for each donor. BMP-2 production was standardized by cell number and reported as nanograms of BMP-2 per 1×10^6 cells per 24 h.

Population Doubling Time

Population doubling time (PDT) was determined based on growth data from P2 to P3. Cell numbers at P2 and P3 as well as number of days between P2 and P3 were recorded and PDT was determined using the doubling time/exponential growth equation $T_d = (t_2 - t_1) \cdot \frac{\ln(2)}{\ln\left(\frac{q_2}{q_1}\right)}$.

where t2-t1= time in days from P2 to P3, and q2/q1 represent cell numbers at P3 and P2, respectively.(43)

Mineralization Assay

Osteogenic differentiation profiles of non-transduced and LV-TSTA-BMP-2 transduced ASCs were assessed using alizarin red (AR) staining and quantification with spectrophotometry.(23, 44)

2×10^5 non-transduced and transduced cells were plated in duplicate in 6-well plates and maintained in osteogenic media for 14 days prior to AR staining. Osteogenic induction media included DMEM + 10% FBS + antibiotics/antimycotic along with 10 μM dexamethasone, 50 μg/mL L-ascorbic acid, 10 mM Beta-glycerophosphate. After 14 days, cells were fixed in 10 % formaldehyde for 10 minutes, washed with PBS and stained with 2 % Alizarin Red S solution for 30 minutes at room temperature. Remaining dye was removed with distilled water. AR staining was imaged using an Echo Revolve microscope, Model RVL-100-B2 (Bico, Boston, MA) and stored at −20°C prior to quantification.

For quantification of Alizarin Red S, a colorimetric assay was used. 800 μl of 10 % acetic acid was added to stained cells in each well of the 6-well plates and incubated at room temperature for 30 minutes with gentle shaking. Cells were scraped and transferred to 1.5 mL eppendorf tubes and subsequently incubated at 85°C for 10 minutes. After centrifugation

at 20,000 *g* for 5 minutes, 500 μL of supernatant was transferred to a new tube. The supernatant was neutralized with 10 % ammonium hydroxide, then 150 μL aliquots of the sample were added in triplicates to an opaque-walled clear-bottom 96-well plate. The absorbance was read at 405 nm with a plate reader (Model 680, BioRad, Hercules, CA, USA).

Colony Forming Unit – Fibroblast Assay

Stemness of harvested ASCs, as defined by self-renewal capacity, was assessed by quantification of colony forming unit-fibroblast (CFU-F) assays in both non-transduced and LV-TSTA-BMP-2 transduced ASCs. 24 hours after transduction (or 48 hours after plating for non-transduced cells), cells were trypsinized, filtered with a 70 μm strainer to remove cell clumps, and then counted using an automated cell counter with trypan blue. Cells were then seeded in triplicate at a density of 100 and 200 cells per 10 cm plate. Cells were maintained in culture with standard growth medium for 14 days with media changes every 3–4 days. After 14 days in culture, the cells were washed and stained with crystal violet and colonies were manually enumerated by 3 different readers (KC, MCG, OS). Replicates were averaged for each reader and the data presented for each condition are the mean of the three readers' averages. Inter-rater reliability was assessed with an intraclass correlation coefficient (ICC) with a two-way mixed effects model and was characterized as excellent (≥ 0.9), good (0.75–0.9), fair (0.4–0.75), or poor (< 0.4).(45, 46)

Statistical Analysis

The SPSS software (IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp) was used for analysis of the data. The significance level was set at 0.05. Data are presented as mean and standard deviation and were assessed for normality with the Shapiro-Wilk test prior to further analysis. Cell number at different passages (P0-P3) and population doubling time were compared between patients with and without comorbidities using Mann-Whitney U test. These results were then adjusted for age, sex, BMI, and race to control for any confounding factors using linear regression. Differences in cell number and population doubling time were also evaluated in male versus female patients and patients of different race using Mann-Whitney U tests.

We then evaluated BMP-2 production, mineralization potential, and self-renewal capacity in a subset of patients with and without comorbidities using independent samples t-test and Mann Whitney U test, as applicable. The results were controlled for age, sex, BMI, race, smoking, and prior arthroscopic knee surgery using linear regression. Chi-square tests were used for univariate analysis of the different categorical variables, with Pearson Chi square results or Fisher exact test results (for expected values of less than 5) reported, as applicable. Finally, Pearson correlation analysis was performed to determine the relationship between BMP-2 levels and different studied parameters (age, sex, BMI, CFU-F) as well as number of cells per passage and BMI or age.

RESULTS

Overall, 122 donors were included in the study. All subjects were evaluated for cell yield and growth characteristics. A subset of 44 donors (21 healthy and 23 co-morbid) underwent additional analysis including immunophenotyping and assessment of osteogenic potential.

Patient Demographics

The average donor age was 65.9 ± 8.2 years (range 46 to 80 years old), and the average BMI was 31.5 kg/m^2. 55% of donors were female, and 52% were white (Table 1). Compared to the co-morbid donors, healthy donors were significantly younger (p=0.004) and more likely to be white (p=0.002), but there was no difference in the proportion of male and female patients in each group.

CD Marker Profile of Cells

Immunophenotypic analysis of non-transduced ASCs revealed minimal expression of lineage markers CD34, CD11b, CD19, and HLA-DR (99.78% of cells negative for all lineage markers). Of these lineage negative cells, there was high expression of the previously defined MSC markers CD44, CD73, CD90 (94.36%, 96.84%, and 94.76%, respectively (Table 2). This combination of CD markers is consistent with the MSC phenotype based on the criteria established by the ISCT on the minimal set of specific surface antigen expression to characterize MSCs.(38)

Assessment of Cell Yield and Proliferation in the Overall Cohort

ASC yield and growth data are listed in Table 3. The average volume of harvested adipose tissue from the infrapatellar fat pad was 11.8 ± 4.7 mL. There was no statistically significant correlation between health status, age, or race and ASC yield or proliferation, when controlling for confounding factors (data not shown). There was a weak, negative, statistically significant correlation between BMI and number of SVF cells isolated per mL of tissue (r = −.234, p=0.016). No correlations were noted between BMI and cell number at passages 1–3 or population doubling time (PDT).

Isolated SVF/mL of tissue, as well as cell number at each successive passage was statistically significantly higher in females when compared to males (Table 3), though cell growth as measured by PDT was not significantly different between males and females (p=0.50).

Population Doubling Time of LV-TSTA-BMP-2 Transduced ASCs

No differences were noted in PDT of transduced ASCs between healthy and co-morbid donors, when controlling for age, race, sex, and BMI (38.6 vs 40.3 days, adjusted p=0.49) (Table 4).

In vitro BMP-2 Production by LV-TSTA-BMP-2 Transduced ASCs

ELISA confirmed successful transduction of ASCs with LV-TSTA-BMP-2 and abundant BMP-2 production over a 24hr period at 2 days, 7 days, and 14 days post-transduction. ASCs produced 151.8 ± 95.6, 884.9 ± 434.0, and 391.4 ± 282.5 ng of BMP-2 per 1×10^6

cells at 2-, 7-, and 14-days post-transduction, respectively. As expected, non-transduced ASCs did not produce any BMP-2 (0.00 ng).

ASCs from healthy donors produced significantly more BMP-2 at 2 days than co-morbid ASCs when adjusting for confounders (p=0.046, Table 4). However, this difference did not persist at days 7 or 14 post-transduction.

In the adjusted linear regression models, there was no association between sex, race, age or BMI and BMP-2 production at any timepoint.

In vitro Osteogenic Potential of Non-transduced and LV-TSTA-BMP-2 Transduced ASCs

Alizarin red staining demonstrated abundant extracellular calcium deposition in non-transduced and transduced ASCs after two weeks in osteogenic differentiation medium. Quantitative analysis of alizarin red cultures demonstrated significantly higher calcium deposition in non-transduced cells when compared with transduced cells irrespective of health status (0.517 vs 0.329 a.u., OD 405, p=.001, data not shown). However, in both non-transduced and LV-TSTA-BMP-2 transduced ASCs, there were no significant quantitative differences in extracellular matrix calcification when stratified by health status, sex, race, age or BMI (Table 4). A representative image of calcium deposition/mineralization in transduced and non-transduced ASCs is shown in Figure 1.

In Vitro Assessment of Colony Forming Cells in Non-transduced and LV-TSTA-BMP-2 Transduced ASCs

Self-renewal capacity was evaluated at passage 3 in non-transduced ASCs as well as in LV-TSTA-BMP-2 transduced ASCs. Intraclass correlation coefficient revealed excellent inter-rater reliability for colony identification for our three raters (ICC 0.99, 95 % CI = 0.996 to 0.998)

Overall, non-transduced cells produced a greater number of colonies compared to transduced cells when plated at either 100 cells/plate (27.0 vs. 4.4, p<0.001) or 200cells/plate (47.9 vs. 9.6, p<0.001). However, in both transduced and non-transduced cells, there was no association between number of colonies and health status, sex, race, age or BMI at either plating density (Table 4).

DISCUSSION

ASCs are a leading candidate for use in the setting of regional gene therapy for bone repair. They are easy to harvest, with low donor morbidity, and demonstrate robust osteogenic capacity and BMP-2 production following lentiviral transduction with the BMP-2 gene.(23) In order to implement successful and efficient clinical use of an hASC-based gene therapy treatment, it is necessary to understand possible differences in the biologic potential of ASCs harvested from donors of varying age and with a variety of co-morbid conditions.

Our evaluation of data on 122 harvested infrapatellar fat pads revealed no significant differences in ASC yield or growth characteristics based on age, race, or the presence of comorbidities. Our *in vitro* analysis of osteogenic potential in 44 ASC donors with

and without medical comorbidities including diabetes mellitus, coronary artery disease, rheumatoid arthritis, and systemic immunosuppression found no differences, either in transduced or non-transduced ASCs, with respect to: self-renewal capacity as determined by CFU-F assay; osteogenic activity as determined by mineralization quantification; or growth kinetics as determined by population doubling time, after controlling for age, gender, and race.

Our results did demonstrate a small but statistically significant decrease in BMP-2 production 2 days post-transduction in cells from donors with comorbidities, compared with those from healthy donors. However, the difference was no longer significant at 7- and 14-days post-transduction. This finding is crucial considering that prior preclinical studies using hASCs in long bone critical sized defect models have demonstrated that hASC BMP-2 production is necessary for bone healing, while nontransduced ASCs alone are incapable of complete defect healing.(13) Furthermore, we have previously demonstrated that higher doses of BMP-2-producing transduced MSCs correlate with increased healing and bone volumes in a rat critical femoral defect model.(10) Lastly, given that bone regeneration takes place over a course of several weeks to months, and that lentiviral-transduced BMP-2 producing MSCs have been shown to persist in bone defects up to 8 weeks in preclinical models, we do not believe that the small difference in BMP-2 production at 2 days would have a clinically significant effect on bone healing.(42, 47) Taken together with the results of our study, these findings suggest that regardless of donor age, sex, race, and presence of comorbidities, hASCs transduced with an LV-BMP-2 vector maintain similar bone regenerative capacity and are a promising source for autologous cell-based gene therapy applications for bone repair. However, *in vivo* studies are needed to evaluate any potential impact of ASC donor age and comorbidities on bone healing.

With respect to sex-based differences, we found a statistically significantly higher ASC yield and cell numbers at all consecutive passages from female donors compared to males. To our knowledge, a relationship between gender and ASC proliferation has not been previously reported. However, prior investigation of sex-based differences in ASCs has demonstrated differences on a transcriptomic level which have the potential to influence proliferation and differentiation.(48) *In vitro* studies using both non-human and human ASCs, as well as human muscle-derived MSCs, have all shown a tendency toward increased osteogenic potential of male compared to female MSCs.(49–52) It is worth noting that the prior *in vitro* studies using human MSC donors evaluated a small number of donors (6–8 total), and that our study evaluating *in vitro* mineralization of transduced and non-transduced ASCs from 44 separate donors did not demonstrate any sex-based differences in osteogenic potential. Our finding of sex-based differential cell proliferation did not correlate in our study with any *in vitro* differences in BMP-2 production or osteogenic differentiation. This finding could have potential implications for tissue culture-related time and resources in the setting of *ex vivo* regional gene therapy strategies, especially if high cell numbers are required.

Our finding of a weak but statistically significant negative correlation between donor BMI and initial cell yield has uncertain clinical significance, especially considering that cell numbers at successive passages and overall growth kinetics as determined by PDT did not demonstrate a correlation with BMI. Of note, a prior study by Frazier et. al evaluated

proliferation of ASCs from the subcutaneous fat of 12 white females of varying ages, and demonstrated a negative correlation between BMI and both cell proliferation and osteogenic potential(53).

While our results did not demonstrate any significant correlations between ASC osteogenic potential or multipotency and donor characteristics such as health status, age, sex, or race, we did note significantly increased mineralization in non-transduced ASCs after two weeks in osteogenic media when compared with transduced cells, as well as decreased colony formation in transduced ASCs compared with non-transduced counterparts. Our finding of decreased mineralization is consistent with a recent study by Toth et al. which evaluated the effect of inducible BMP-2 expression in dental pulp stem cells on matrix mineralization(54). These authors noted a similar decrease in matrix mineralization and ALP activity associated with BMP-2 expression, which they attributed to BMP-2 mediated increased expression of Runx2 as well as BMP antagonist *noggin*. Our finding of decreased colony formation in transduced ASCs follows logically as BMP-2 production would theoretically drive osteoblastic differentiation, manifesting in fewer colonies on CFU assay.

Our study does have limitations. First, adipose tissue was harvested from a single anatomic location, which decreases the generalizability of our results despite likely decreasing variability and increasing internal validity. Differences in stem cell behavior associated with specific cell harvest location (i.e. subcutaneous versus visceral adipose tissue) have been demonstrated and warrant continued investigation.(55, 56) Furthermore, it has been hypothesized that the infrapatellar fat pad plays a role in the pathophysiology of osteoarthritis and prior studies have demonstrated phenotypic changes in ASCs derived from infrapatellar fat pads of osteoarthritic knees.(57) This raises the concern that ASCs even from our “healthy” donors, while serving as good internal controls for the comparison of systemic comorbidities on ASC properties, are derived from a diseased (osteoarthritic) source. Importantly, for the purposes of cell-based therapies and gene therapy, several studies have demonstrated comparable multi-differentiation potential of infrapatellar fat pad-derived ASCs from patients with OA and those from healthy patients without OA, and are still considered a promising source of ASCs for tissue regeneration.(58–60) An additional limitation is that, due to the natural history of osteoarthritis and the typical demographic of patients undergoing TKA, our study only included three patients under age 50. We chose the infrapatellar fat pad as the source as this is normally removed and discarded in TKA and therefore we did not subject patients to an increase in donor morbidity. However, it is important to consider that prior studies have noted enhanced biological activity in ASCs from younger donors compared to those from older donors (e.g. < 30 versus > 50 years old).(35) Nonetheless, transduced ASCs in this study produced an abundant amount of BMP-2. Another limitation is that we placed patients into healthy and co-morbid groups. It is possible that certain comorbidities have a greater impact than others on the biologic activity of ASCs. Finally, the results of our *in vitro* analyses apply specifically to lentiviral-transduced cells engineered to overexpress BMP-2 for the purposes of bone tissue regeneration. For example, possible effects of MSC donor co-morbidities on adipogenic or chondrogenic potential were not evaluated in the present study. Thus, caution must be exercised when generalizing these results to other gene therapy applications using different vectors or target genes and tissues.

In conclusion, in one of the largest studies evaluating ASC functionality and osteogenic potential, we found that donor age and co-morbid conditions have a limited role in ASC yield, growth characteristics, self-renewal capacity, and osteogenic potential. Similarly, the presence of donor comorbidities did not affect the *in vitro* functionality of ASCs transduced to overexpress BMP-2. Regardless of donor age, sex, race, or health status, our findings suggest that human ASCs harvested from infrapatellar fat pads maintain similar bone regenerative capacity. Collectively, these *in vitro* results are in support of ASCs as a promising resource for autologous cell-based gene therapy applications for bone repair. Future studies should investigate the impact of ASC donor age and comorbidities on *in vivo* bone formation.

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DATA AVAILABILITY STATEMENT:

Additional data are available from the corresponding author upon reasonable request.

REFERENCES

1. Collon K, Gallo MC, Lieberman JR. Musculoskeletal tissue engineering: Regional gene therapy for bone repair. Biomaterials. 2021;275:120901. [PubMed: 34091300]
2. Giannoudis PV, Einhorn TA, Marsh D. Fracture healing: the diamond concept. Injury. 2007;38 Suppl 4:S3–6.
3. Baldwin P, Li DJ, Auston DA, Mir HS, Yoon RS, Koval KJ. Autograft, Allograft, and Bone Graft Substitutes: Clinical Evidence and Indications for Use in the Setting of Orthopaedic Trauma Surgery. J Orthop Trauma. 2019;33(4):203–13. [PubMed: 30633080]
4. Ahlmann E, Patzakis M, Roidis N, Shepherd L, Holtom P. Comparison of anterior and posterior iliac crest bone grafts in terms of harvest-site morbidity and functional outcomes. J Bone Joint Surg Am. 2002;84(5):716–20. [PubMed: 12004011]
5. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng. 2001;7(2):211–28. [PubMed: 11304456]
6. Lieberman JR, Daluiski A, Stevenson S, Wu L, McAllister P, Lee YP, et al. The effect of regional gene therapy with bone morphogenetic protein-2-producing bone-marrow cells on the repair of segmental femoral defects in rats. J Bone Joint Surg Am. 1999;81(7):905–17. [PubMed: 10428121]
7. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science. 1999;284(5411):143–7. [PubMed: 10102814]
8. Chen TL, Bates RL, Dudley A, Hammonds RG Jr., Amento EP. Bone morphogenetic protein-2b stimulation of growth and osteogenic phenotypes in rat osteoblast-like cells: comparison with TGF-beta 1. J Bone Miner Res. 1991;6(12):1387–93. [PubMed: 1792947]
9. Cuomo AV, Virk M, Petrigliano F, Morgan EF, Lieberman JR. Mesenchymal stem cell concentration and bone repair: potential pitfalls from bench to bedside. J Bone Joint Surg Am. 2009;91(5):1073–83. [PubMed: 19411455]

10. Ihn H, Kang H, Iglesias B, Sugiyama O, Tang A, Hollis R, et al. Regional Gene Therapy with Transduced Human Cells: The Influence of “Cell Dose” on Bone Repair. Tissue Eng Part A. 2021;27(21–22):1422–33. [PubMed: 33882718]
11. Feeley BT, Conduah AH, Sugiyama O, Krenek L, Chen IS, Lieberman JR. In vivo molecular imaging of adenoviral versus lentiviral gene therapy in two bone formation models. J Orthop Res. 2006;24(8):1709–21. [PubMed: 16788987]
12. Musgrave DS, Bosch P, Lee JY, Pelinkovic D, Ghivizzani SC, Whalen J, et al. Ex vivo gene therapy to produce bone using different cell types. Clin Orthop Relat Res. 2000(378):290–305.
13. Vakhshori V, Bougioukli S, Sugiyama O, Kang HP, Tang AH, Park SH, et al. Ex vivo regional gene therapy with human adipose-derived stem cells for bone repair. Bone. 2020;138:115524. [PubMed: 32622870]
14. Pelled G, Sheyn D, Tawackoli W, Jun DS, Koh Y, Su S, et al. BMP6-Engineered MSCs Induce Vertebral Bone Repair in a Pig Model: A Pilot Study. Stem Cells Int. 2016;2016:6530624. [PubMed: 26770211]
15. Miyazaki M, Zuk PA, Zou J, Yoon SH, Wei F, Morishita Y, et al. Comparison of human mesenchymal stem cells derived from adipose tissue and bone marrow for ex vivo gene therapy in rat spinal fusion model. Spine (Phila Pa 1976). 2008;33(8):863–9. [PubMed: 18404105]
16. Friedenstein AJ, Petrakova KV, Kurolesova AI, Frolova GP. Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. Transplantation. 1968;6(2):230–47. [PubMed: 5654088]
17. Via AG, Frizziero A, Oliva F. Biological properties of mesenchymal Stem Cells from different sources. Muscles Ligaments Tendons J. 2012;2(3):154–62. [PubMed: 23738292]
18. Hass R, Kasper C, Böhm S, Jacobs R. Different populations and sources of human mesenchymal stem cells (MSC): A comparison of adult and neonatal tissue-derived MSC. Cell Commun Signal. 2011;9:12. [PubMed: 21569606]
19. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, et al. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell. 2002;13(12):4279–95. [PubMed: 12475952]
20. Liao HT, Chen CT. Osteogenic potential: Comparison between bone marrow and adipose-derived mesenchymal stem cells. World J Stem Cells. 2014;6(3):288–95. [PubMed: 25126378]
21. Zhu Y, Liu T, Song K, Fan X, Ma X, Cui Z. Adipose-derived stem cell: a better stem cell than BMSC. Cell Biochem Funct. 2008;26(6):664–75. [PubMed: 18636461]
22. Dragoo JL, Choi JY, Lieberman JR, Huang J, Zuk PA, Zhang J, et al. Bone induction by BMP-2 transduced stem cells derived from human fat. J Orthop Res. 2003;21(4):622–9. [PubMed: 12798061]
23. Bougioukli S, Sugiyama O, Pannell W, Ortega B, Tan MH, Tang AH, et al. Gene Therapy for Bone Repair Using Human Cells: Superior Osteogenic Potential of Bone Morphogenetic Protein 2-Transduced Mesenchymal Stem Cells Derived from Adipose Tissue Compared to Bone Marrow. Hum Gene Ther. 2018;29(4):507–19. [PubMed: 29212377]
24. Maredziak M, Marycz K, Tomaszewski KA, Kornicka K, Henry BM. The Influence of Aging on the Regenerative Potential of Human Adipose Derived Mesenchymal Stem Cells. Stem Cells Int. 2016;2016:2152435. [PubMed: 26941800]
25. Kornicka K, Marycz K, Tomaszewski KA, Marędziak M, Śmieszek A. The Effect of Age on Osteogenic and Adipogenic Differentiation Potential of Human Adipose Derived Stromal Stem Cells (hASCs) and the Impact of Stress Factors in the Course of the Differentiation Process. Oxid Med Cell Longev. 2015;2015:309169. [PubMed: 26246868]
26. Dufrane D Impact of Age on Human Adipose Stem Cells for Bone Tissue Engineering. Cell Transplant. 2017;26(9):1496–504. [PubMed: 29113460]
27. Mantovani C, Raimondo S, Haneef MS, Geuna S, Terenghi G, Shawcross SG, et al. Morphological, molecular and functional differences of adult bone marrow- and adipose-derived stem cells isolated from rats of different ages. Exp Cell Res. 2012;318(16):2034–48. [PubMed: 22659169]
28. Beane OS, Fonseca VC, Cooper LL, Koren G, Darling EM. Impact of aging on the regenerative properties of bone marrow-, muscle-, and adipose-derived mesenchymal stem/stromal cells. PLoS One. 2014;9(12):e115963. [PubMed: 25541697]

29. de Girolamo L, Lopa S, Arrigoni E, Sartori MF, Baruffaldi Preis FW, Brini AT. Human adipose-derived stem cells isolated from young and elderly women: their differentiation potential and scaffold interaction during in vitro osteoblastic differentiation. Cytotherapy. 2009;11(6):793–803. [PubMed: 19878065]
30. Payr S, Schuseil T, Unger M, Seeliger C, Tiefenboeck T, Balmayor ER, et al. Effect of donor age and 3D-cultivation on osteogenic differentiation capacity of adipose-derived mesenchymal stem cells. Sci Rep. 2020;10(1):10408. [PubMed: 32591595]
31. Chen HT, Lee MJ, Chen CH, Chuang SC, Chang LF, Ho ML, et al. Proliferation and differentiation potential of human adipose-derived mesenchymal stem cells isolated from elderly patients with osteoporotic fractures. J Cell Mol Med. 2012;16(3):582–93. [PubMed: 21545685]
32. Dzhoyashvili NA, Efimenko AY, Kochegura TN, Kalinina NI, Koptelova NV, Sukhareva OY, et al. Disturbed angiogenic activity of adipose-derived stromal cells obtained from patients with coronary artery disease and diabetes mellitus type 2. J Transl Med. 2014;12:337. [PubMed: 25491476]
33. Espagnolle N, Hebraud B, Descamps JG, Gadelorge M, Joubert MV, Ferreira LDS, et al. Functional Comparison between Healthy and Multiple Myeloma Adipose Stromal Cells. Stem Cells Int. 2020;2020:4173578. [PubMed: 32215016]
34. Frazier TP, Gimble JM, Devay JW, Tucker HA, Chiu ES, Rowan BG. Body mass index affects proliferation and osteogenic differentiation of human subcutaneous adipose tissue-derived stem cells. BMC Cell Biol. 2013;14:34. [PubMed: 23924189]
35. Yang HJ, Kim KJ, Kim MK, Lee SJ, Ryu YH, Seo BF, et al. The stem cell potential and multipotency of human adipose tissue-derived stem cells vary by cell donor and are different from those of other types of stem cells. Cells Tissues Organs. 2014;199(5–6):373–83. [PubMed: 25823468]
36. Zhang M, Li Y, Rao P, Huang K, Luo D, Cai X, et al. Blockade of receptors of advanced glycation end products ameliorates diabetic osteogenesis of adipose-derived stem cells through DNA methylation and Wnt signalling pathway. Cell Prolif. 2018;51(5):e12471. [PubMed: 30014569]
37. Zhu M, Heydarkhan-Hagvall S, Hedrick M, Benhaim P, Zuk P. Manual isolation of adipose-derived stem cells from human lipoaspirates. J Vis Exp. 2013(79):e50585. [PubMed: 24121366]
38. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006;8(4):315–7. [PubMed: 16923606]
39. Alaee F, Bartholomae C, Sugiyama O, Virk MS, Drissi H, Wu Q, et al. Biodistribution of LV-TSTA transduced rat bone marrow cells used for “ex-vivo” regional gene therapy for bone repair. Curr Gene Ther. 2015;15(5):481–91. [PubMed: 26264707]
40. Iyer M, Wu L, Carey M, Wang Y, Smallwood A, Gambhir SS. Two-step transcriptional amplification as a method for imaging reporter gene expression using weak promoters. Proc Natl Acad Sci U S A. 2001;98(25):14595–600. [PubMed: 11734653]
41. Virk MS, Sugiyama O, Park SH, Gambhir SS, Adams DJ, Drissi H, et al. “Same day” ex-vivo regional gene therapy: a novel strategy to enhance bone repair. Mol Ther. 2011;19(5):960–8. [PubMed: 21343916]
42. Virk MS, Conduah A, Park SH, Liu N, Sugiyama O, Cuomo A, et al. Influence of short-term adenoviral vector and prolonged lentiviral vector mediated bone morphogenetic protein-2 expression on the quality of bone repair in a rat femoral defect model. Bone. 2008;42(5):921–31. [PubMed: 18295562]
43. Zhan XS, El-Ashram S, Luo DZ, Luo HN, Wang BY, Chen SF, et al. A Comparative Study of Biological Characteristics and Transcriptome Profiles of Mesenchymal Stem Cells from Different Canine Tissues. Int J Mol Sci. 2019;20(6).
44. Gregory CA, Gunn WG, Peister A, Prockop DJ. An Alizarin red-based assay of mineralization by adherent cells in culture: comparison with cetylpyridinium chloride extraction. Anal Biochem. 2004;329(1):77–84. [PubMed: 15136169]
45. Cicchetti DV, Sparrow SA. Developing criteria for establishing interrater reliability of specific items: applications to assessment of adaptive behavior. Am J Ment Defic. 1981;86(2):127–37. [PubMed: 7315877]

46. Shrout PE, Fleiss JL. Intraclass correlations: uses in assessing rater reliability. Psychol Bull. 1979;86(2):420–8. [PubMed: 18839484]
47. Alaee FB C; Sugiyama O; Virk MS; Drissi H; Wu Q; Schmidt M; Lieberman JR. Biodistribution of LV-TSTA Transduced Rat Bone Marrow Cells Used for “Ex-vivo” Regional Gene Therapy for Bone Repair. Current Gene Therapy. 2015;15:481–91. [PubMed: 26264707]
48. Bianconi E, Casadei R, Frabetti F, Ventura C, Facchin F, Canaider S. Sex-Specific Transcriptome Differences in Human Adipose Mesenchymal Stem Cells. Genes (Basel). 2020;11(8).
49. Aksu AE, Rubin JP, Dudas JR, Marra KG. Role of gender and anatomical region on induction of osteogenic differentiation of human adipose-derived stem cells. Ann Plast Surg. 2008;60(3):306–22. [PubMed: 18443514]
50. Dudas JR, Losee JE, Penascino VM, Smith DM, Cooper GM, Mooney MP, et al. Leporine-derived adipose precursor cells exhibit in vitro osteogenic potential. J Craniofac Surg. 2008;19(2):360–8. [PubMed: 18362712]
51. Ogawa R, Mizuno H, Watanabe A, Migita M, Hyakusoku H, Shimada T. Adipogenic differentiation by adipose-derived stem cells harvested from GFP transgenic mice-including relationship of sex differences. Biochem Biophys Res Commun. 2004;319(2):511–7. [PubMed: 15178436]
52. Scibetta AC, Morris ER, Liebowitz AB, Gao X, Lu A, Philippon MJ, et al. Characterization of the chondrogenic and osteogenic potential of male and female human muscle-derived stem cells: Implication for stem cell therapy. J Orthop Res. 2019;37(6):1339–49. [PubMed: 30667562]
53. Frazier TP, Gimble JM, Devay JW, Tucker HA, Chiu ES, Rowan BG. Body mass index affects proliferation and osteogenic differentiation of human subcutaneous adipose tissue-derived stem cells. BMC Cell Biology. 2013;14(34).
54. Toth F, Gall JM, Tozser J, Hegedus C. Effect of inducible bone morphogenetic protein 2 expression on the osteogenic differentiation of dental pulp stem cells in vitro. Bone. 2020;132:115214. [PubMed: 31884130]
55. Chen R, Ren L, Cai Q, Zou Y, Fu Q, Ma Y. The role of epigenetic modifications in the osteogenic differentiation of adipose-derived stem cells. Connect Tissue Res. 2019;60(6):507–20. [PubMed: 31203665]
56. Ritter A, Friemel A, Roth S, Kreis NN, Hoock SC, Safdar BK, et al. Subcutaneous and Visceral Adipose-Derived Mesenchymal Stem Cells: Commonality and Diversity. Cells. 2019;8(10).
57. Stocco E, Barbon S, Piccione M, Belluzzi E, Petrelli L, Pozzuoli A, et al. Infrapatellar Fat Pad Stem Cells Responsiveness to Microenvironment in Osteoarthritis: From Morphology to Function. Front Cell Dev Biol. 2019;7:323. [PubMed: 31921840]
58. Greif DN, Kouroupis D, Murdock CJ, Griswold AJ, Kaplan LD, Best TM, et al. Infrapatellar Fat Pad/Synovium Complex in Early-Stage Knee Osteoarthritis: Potential New Target and Source of Therapeutic Mesenchymal Stem/Stromal Cells. Front Bioeng Biotechnol. 2020;8:860. [PubMed: 32850724]
59. Liu Y, Buckley CT, Almeida HV, Mulhall KJ, Kelly DJ. Infrapatellar fat pad-derived stem cells maintain their chondrogenic capacity in disease and can be used to engineer cartilaginous grafts of clinically relevant dimensions. Tissue Eng Part A. 2014;20(21–22):3050–62. [PubMed: 24785365]
60. Pires de Carvalho P, Hamel KM, Duarte R, King AG, Haque M, Dietrich MA, et al. Comparison of infrapatellar and subcutaneous adipose tissue stromal vascular fraction and stromal/stem cells in osteoarthritic subjects. J Tissue Eng Regen Med. 2014;8(10):757–62. [PubMed: 22807102]

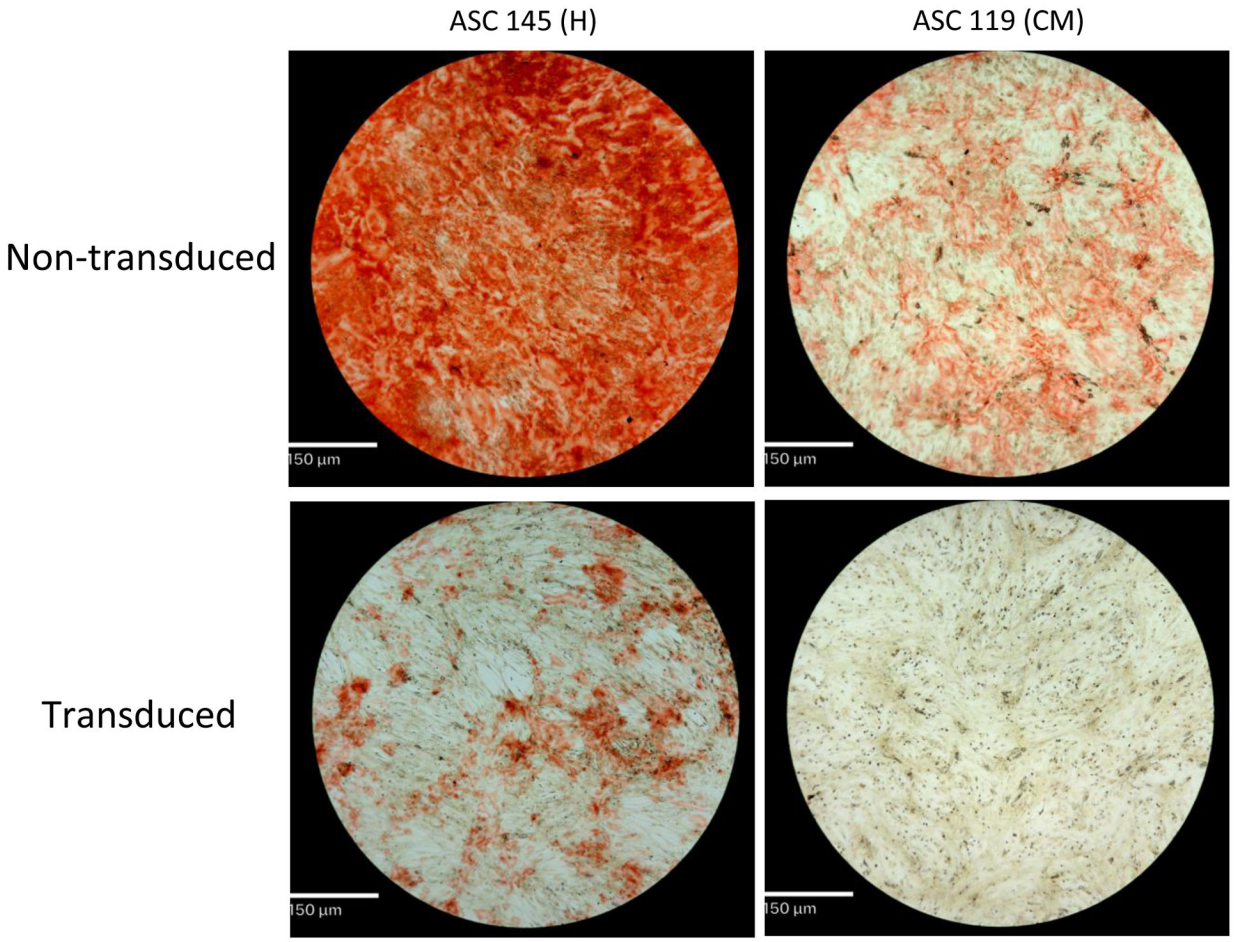

Figure 1.

Representative images of calcium deposition/matrix mineralization in transduced and non-transduced cells. On average, transduced ASCs demonstrated less mineral deposition than non-transduced ASCs. (H)=Healthy; (CM)=Co-Morbid

Table 1:

Subject Demographics

	Overall Cohort (n=122)		Sub Cohort (n=44)				
			Healthy (n=21)		Co-Morbid (n=23)		
	Mean	SD	Mean	SD	Mean	SD	P value
Age	65.9	8.2	63.4	7.9	70.3	7.1	**0.004**
Sex	67 F, 55 M		11 F, 10 M		12 F, 11 M		0.9
Race	63 W, 40 NW, 19 NR		18 W, 3 NW		9 W, 14 NW		**0.002**
BMI (kg/m^2)	31.5	6.8	29.9	6.7	32.6	6.7	0.18

F=Female, M=Male, W=White, NW=Non-White, NR=Not Reported

Table 2:

Confirmation of MSC Immunophenotype in Harvested ASCs

	CD90+	CD44+	CD73+
Avg. % of lin. Neg. cells ± SD	94.76 ± 3.41	94.36 ± 4.67	96.84 ± 3.24

Table 3:

Non-transduced ASC Growth Characteristics

		SVF*	P1*	P2*	P3*	PDT (n=91)**
Health Status	Healthy	0.64 ± 0.58	0.45 ± 0.51	1.16 ± 1.25	2.97 ± 3.60	7.6 ± 4.5
	Co-morbid	0.64 ± 0.91	0.45 ± 0.73	1.16 ± 1.48	3 ± 4.67	8.9 ± 5
	P value	0.22	0.19	0.70	0.62	0.11
	Adjusted P Value	0.96	0.89	0.93	0.57	0.13
Sex	Male	0.51 ± 0.48	0.36 ± 0.41	0.89 ± 0.88	1.98 ± 1.75	8.07 ± 4.05
	Female	.75 ± 0.81	0.53 ± 0.68	1.41 ± 1.56	3.86 ± 4.98	7.82 ± 5.24
	P value	0.07	0.09	0.01	0.02	0.80
	Adjusted P Value	**0.01**	**0.03**	**0.01**	**0.03**	0.50
Race	White	0.65 ± 0.57	0.49 ± 0.53	1.18 ± 1.33	3.20 ± 3.95	7.76 ± 2.87
	Non-White	0.56 ± .53	0.36 ± 0.30	1.11 ± 0.68	2.70 ± 2.14	7.02 ± 2.36
	P value	0.18	0.29	0.45	1.00	0.25
	Adjusted P Value	0.38	0.21	0.95	0.95	0.22

PDT= Population Doubling Time, SVF=Stromal Vascular Fraction.

*Values represent number of cells $\times 10^6$ per mL of adipose tissue.

**Values represent days.

Table 4:

Growth, In Vitro Osteogenic Potential, and Stemness by Health Status

	Healthy	Co-morbid	P Value	Adjusted P Value*
PDT Transduced (days)	38.64	40.31	0.45	0.49
BMP-2 2d (ng per 1×10^6 cells)	187.19	119.46	0.04	**0.05**
BMP-2 7d (ng per 1×10^6 cells)	905.06	866.41	0.67	0.75
BMP-2 14d (ng per 1×10^6 cells)	477.35	312.85	0.02	0.06
AR Nontransduced (OD 405)	0.56	0.48	0.42	0.64
AR Transduced (OD 405)	0.47	0.20	0.12	0.13
CFU-F Transduced[a]	3.93	4.78	0.33	0.54
CFU-F Transduced[b]	8.98	10.14	0.50	0.91
CFU-F Nontransduced[a]	26.19	27.74	0.71	0.49
CFU-F Nontransduced[b]	44.95	50.64	0.40	0.23

Adjusted for race, age, BMI, sex, smoking, prior arthroscopic procedures. PDT= Population Doubling Time, AR= Alizarin Red, CFU-F= Fibroblast Colony Forming Unit.

[a]Cells were plated at a density of 100 per 10 cm plate.

[b]Cells were plated at a density of 200 per 10 cm plate.