Tissue Harvest by Means of Suction-Assisted or Third-Generation Ultrasound-Assisted Lipoaspiration Has No Effect on Osteogenic Potential of Human Adipose-Derived Stromal Cells

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Background: Human adipose-derived stromal cells readily undergo osteogenic differentiation in vitro and in vivo. Thus, interest in their potential role in skeletal tissue engineering continues to escalate. Very little is known regarding the effects that energy delivered by means of third-generation ultrasound-assisted lipoaspiration may have on the osteogenic potential of these cells. The authors investigated whether differences in adipose-derived stromal cell yield, and the in vitro proliferation and osteogenic potential of these cells obtained by suction-assisted lipoaspiration or third-generation ultrasound-assisted lipoaspiration, exist.

Methods: Adipose-derived stromal cells were harvested from lipoaspiration specimens of patients undergoing elective suction-assisted lipoaspiration and third-generation ultrasound-assisted lipoaspiration. Harvested cells were seeded to evaluate proliferative capacity and in vitro osteogenic potential. Alkaline phosphatase and alizarin red staining were performed to evaluate early and terminal osteogenic differentiation, respectively. Quantitative real-time polymerase chain reaction analysis was used to examine osteogenic gene expression patterns of *RUNX2/CFBA1* (early differentiation) and osteocalcin (late differentiation).

Results: No significant differences in the proliferative capacity ($n = 3$), alkaline phosphatase staining ($n = 3$), or extracellular matrix mineralization ($n = 3$) of suction-assisted lipoaspiration– or third-generation ultrasound-assisted lipoaspiration–derived cells were appreciated. Transcript levels of markers of early and terminal osteogenic differentiation were not significantly different ($n = 3$).

Conclusions: These findings suggest that exposure of adipose-derived stromal cells to ultrasound energy during tissue harvest by means of third-generation ultrasound-assisted lipoaspiration does not impart a negative consequence toward their proliferative capacity or osteogenic potential. Thus, the cells harvested using third-generation ultrasound-assisted lipoaspiration are comparable to those obtained by means of suction-assisted lipoaspiration for use in the study of osteogenic differentiation and skeletal tissue engineering. (*Plast. Reconstr. Surg.* 124: 65, 2009.)

The ability of adipose-derived stromal cells to differentiate along an osteogenic lineage in vitro and to contribute to bone formation in vivo has spawned great interest in their potential utility in skeletal tissue engineering applications.[1,2] Thus, significant enthusiasm has been engendered to pursue a deeper understanding of the cellular and molecular biology underlying the osteogenic potential of this cell population, as the need for novel skeletal reconstructive modalities has never been greater. The burden placed on the U.S. health care system secondary to the treatment of skeletal deficits is significant and expanding.[3] This, paired with the plethora of shortcomings accompanying current skeletal reconstructive modalities that use autogenous and allogeneic bone

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grafting and alloplastic materials, still the mainstays of intervention for skeletal deficits, has engendered enthusiasm to further develop our understanding of the osteogenic potential of adipose-derived stromal cells.[4–6]

In addition to the aforementioned driving forces, the advantages that adipose-derived stromal cells possess over bone marrow–derived stromal cells for use in skeletal tissue engineering applications have further stimulated their investigation. Compared with bone marrow–derived stromal cells, adipose-derived stromal cells can be harvested in large numbers with relatively low donor morbidity. Furthermore, the similar proliferative capacity and osteogenic potential in vitro, and the ability to heal calvarial defects in vivo, of adipose-derived stromal cells compared with bone marrow–derived stromal cells is well documented.[2,7–9] Collectively, these factors make adipose-derived stromal cells ideal for use in the setting of a bedside tissue-engineering strategy.

However, as investigation into the osteogenic potential of human adipose-derived stromal cells continues to escalate, a multitude of confounding variables have been identified that were not previously relevant to the study of murine adipose-derived stromal cells. Numerous studies addressing the effects of various facets of postharvest tissue processing and storage on the proliferative capacity and differentiative potential of adipose-derived stromal cells have been reported on in the literature.[10–13] However, a paucity of commentary has been put forth regarding the potential effects posed by the type of lipoaspiration procedure implemented on these cellular characteristics. Until recently, research investigating adipose-derived stromal cells was performed on lipoaspiration specimens obtained principally by means of suction-assisted lipoaspiration, as this has been the mainstay of surgeons performing lipoplasty since first introduced by Arpad and Giorgio Fisher in the 1970s.[14] In this technique, subcutaneous adipose tissue is removed through mechanical disruption followed by evacuation with a suction cannula. However, with the advent of new technology, the process of lipoaspiration has continued to evolve. Zocchi first developed ultrasound-assisted lipoaspiration in the 1980s, which implemented the use of ultrasound energy to selectively emulsify low-density adipocytes by means of micromechanical, thermal, and microcavitational effects before suction evacuation.[14,15] In concept, the potential clinical gains to be appreciated by both patients and surgeons through the implementation of this technology are attractive, and evidence derived from recent investigations to support this potential is accumulating. Ultrasound-assisted lipoaspiration has been touted by its proponents for improving on traditional lipoaspiration techniques by affording the patient benefits of being less traumatic to treated tissues through the application of energy selective for adipocytes, and being particularly efficacious in the treatment of dense, fibrous anatomical locations.[16,17] At the same time, investigations by Karmo et al. and Kenkel et al. have demonstrated that comparable, if not reduced, levels of blood loss result from ultrasound-assisted lipoaspiration relative to suction-assisted lipoaspiration.[18,19] In addition to these patient benefits, progress in the conduct of lipoaspiration arising from technical refinements in ultrasound-assisted lipoaspiration equipment have been reported by operating surgeons.[14,16] However, the technique is not without its drawbacks. Complications can result from thermal injury and seroma formation, arising from excessive exposure to ultrasound energy largely attributable to technical error on the part of the operator.[20,21] These complications have necessitated the refinement of ultrasound-assisted lipoaspiration technology, such as seen in new third-generation ultrasound-assisted lipoaspiration devices. Modifications now allow for the disruption of adipose tissue through pulsed application of reduced amounts of ultrasound energy.[14] Further concerns were vocalized in the 1990s regarding the potential adverse long-term effects of ultrasound on treated tissues arising from factors including sonoluminescence, free radical production through cavitation, and thermal effects.[22] Stemming from these concerns, the Aesthetic Society Education and Research Foundation established a safety panel to investigate their validity. The panel's findings, and those of other investigators, established that concern over long-term tissue effects arising from the above factors is unfounded.[19,21,23]

The true benefits appreciated by patients treated with ultrasound-assisted lipoaspiration compared with alternative lipoplasty techniques remain to be elucidated. However, the adaptation of ultrasound-assisted lipoaspiration in the clinical arena is expanding at an exponential rate. As such, an ever-increasing number of lipoaspiration specimens obtained for the study of skeletal tissue engineering are being procured by means of this technique, containing adipose-derived stromal cells that have been exposed to ultrasound energy.[16,24] This trend imparts significant implications toward the development of translational reconstructive therapies using adipose-derived stromal cell–based skeletal tissue engineering techniques, as the effects of ultrasound energy delivered by means of third-generation ultrasound-assisted lipoaspiration devices on the osteogenic potential of adipose-derived stromal cells is yet to

be elucidated. Thus, in this study, we investigated whether differences in proliferative capacity, in vitro osteogenic potential, and expression of osteogenic genes exist between adipose-derived stromal cells obtained by means of suction-assisted lipoaspiration versus third-generation ultrasound-assisted lipoaspiration. Given the nature of micromechanical forces, thermal effects, and phenomena including sonochemical production resulting from exposure to ultrasound energy previously established, we hypothesized that no significant differences in the osteogenic potential of adipose-derived stromal cells harvested by means of suction-assisted lipoaspiration or third-generation ultrasound-assisted lipoaspiration would be discernable. Information gleaned from studying the effects of the adipose harvest procedure on the ability of adipose-derived stromal cells to differentiate down osteogenic lineages will provide direction toward how tissue specimens are obtained for use in skeletal tissue engineering applications in the future.

MATERIALS AND METHODS

Tissue Procurement

All lipoaspiration specimens were obtained after acquiring informed consent from patients, in accordance with Stanford University Human Institutional Review Board guidelines. All lipoaspiration procedures were performed using the VASER Lipo System (Sound Surgical Technologies, Louisville, Col.). To perform suction-assisted lipoaspiration, aspiration was performed using 3.0- to 5.0-mm hollow cannulas without engaging the ultrasonic amplifier. Third-generation ultrasound-assisted lipoaspiration was performed using 2.9- to 3.7-mm solid probes, delivering energy at a vibration frequency of 36,000 Hz and a wave amplitude ranging from 71 to 76 μm. This translates into vibratory powers ranging from 5 to 12 W.[25] Adipose-derived stromal cells were harvested from the adipose tissue of male and female patients between the ages of 18 and 65 undergoing elective lipoaspiration of the abdomen, flank, and/or thigh region. Adipose-derived stromal cells harvested from anatomical regions included in this study have previously been shown by our laboratory to have no significant differences in osteogenic potential. Participating patients had no prior knowledge or evidence of ongoing systemic disease at the time of operation. Both suction-assisted lipoaspiration and third-generation ultrasound-assisted lipoaspiration specimens were obtained from each patient. All specimens were placed on ice immediately and processed following harvest. The completion of all experimental studies required the harvest of adipose tissue from five patients. Two paired specimens, one harvested by means of suction-assisted lipoaspiration and one by third-generation ultrasound-assisted lipoaspiration, were obtained from one anatomical region of each patient. Suction-assisted lipoaspiration specimens were harvested before third-generation ultrasound-assisted lipoaspiration specimens to avoid exposure of suction-assisted lipoaspiration–derived adipose-derived stromal cells to ultrasound energy. Specimens were collected/deidentified, and processed by independent investigators.

Adipose-Derived Stromal Cell Culture

Immediately after lipoaspiration, adipose specimens were washed sequentially in serial dilutions of dilute povidone-iodine, followed by two phosphate-buffered saline washes of equal volume to each lipoaspiration specimen. Tissues were subsequently digested with an equal volume of 0.075% (weight/volume) type II collagenase in Hank's Balanced Salt Solution at 37°C in a water bath with agitation at 125 rpm for 30 minutes. The collagenase digest was then inactivated by adding an equal volume of standard cell culture growth media [Dulbecco's Modified Eagle Medium plus GlutaMAX (Invitrogen Corp., Carlsbad, Calif.), 10% fetal bovine serum, and 1% penicillin/streptomycin]. The stromal vascular fraction was pelleted by means of centrifugation at 1200 *g* for 5 minutes. The supernatant was then discarded and the cell pellet resuspended and filtered through a 100-μm cell strainer to remove undigested tissue fragments. The cells were pelleted and resuspended in standard cell culture growth media at 37°C in an atmosphere of 5% carbon dioxide. Cells were grown to confluence and passaged with 0.05% trypsin. Media was changed every 3 days. First-passage cells were used for all experiments.

Comparative Analysis of In Vitro Proliferative Capacity

After a brief period of expansion in primary culture, adipose-derived stromal cells were trypsinized and replated for proliferation assays. To evaluate proliferative capacity, 5000 cells/well were seeded in side-by-side, 12-well culture plates. Cell counting was performed in triplicate with a hemacytometer and using trypan blue exclusion, to allow for exclusion of nonviable cells, at 1 day, 3 days, and 7 days after seeding.

Comparative Analysis of In Vitro Osteogenic Capacity

To assay the osteogenic potential of adipose-derived stromal cells, first-passage adipose-derived

stromal cells were again seeded at equal density (100,000 cells/well) in side-by-side, six-well culture plates in triplicate. Adipose-derived stromal cells were cultured in standard cell culture growth media (Dulbecco's Modified Eagle Medium plus GlutaMAX-I, 10% fetal bovine serum, and 1% penicillin/streptomycin) overnight to allow adherence, at which time they were cultured in osteogenic differentiation media (Dulbecco's Modified Eagle Medium, 10% fetal bovine serum, 1% penicillin/streptomycin, 250 μM ascorbic acid, and 10 mM β-glycerol phosphate) for 10 days. Subsequently, adipose-derived stromal cells were assayed for early and terminal osteogenic differentiation by means of alkaline phosphatase staining and alizarin red staining, following 3 and 10 days of osteogenic differentiation, respectively. Stained adipose-derived stromal cells were photographed both grossly and microscopically (5× magnification) to evaluate for differences in osteogenic differentiation. Alizarin red staining of calcified extracellular matrix was quantified as described below. As an additional control for our osteogenic differentiation experimental methods, non–adipose-derived mesenchymal stem cells (bone marrow mesenchymal stem cells; AllCells, Emeryville, Calif.) were subjected to similar experimental osteogenic differentiation conditions, and differentiation was quantified by means of alizarin red staining in a similar fashion as performed for adipose-derived stromal cells.

To perform alkaline phosphatase staining, osteogenic differentiation media was removed and cells were washed twice with phosphate-buffered saline to remove any residual media. Cells were fixed with a mixture of 40% citrate working solution [2% citrate (volume/volume)] and 60% acetone for 30 seconds. Cells were washed briefly with double distilled water to remove fixative solution. Next, staining was performed with diazonium salt solution [20% fast violet B salt (weight/volume)/4% naphthol (volume/volume)] for 30 minutes at room temperature while shielded from light. Staining solution was aspirated; cells were rinsed briefly with double-distilled water to remove any excess staining solution and subsequently photographed grossly and microscopically.

Quantification of alizarin red staining was performed as previously described by Wan et al.[26] In short, cells were fixed with 100% ethanol and incubated for 60 minutes in 0.02% (weight/volume) alizarin red solution (pH, 6.36 to 6.40). A brief wash with double-distilled water, followed by serial washes with phosphate-buffered saline, was performed to remove excess staining solution. Spectrophotometric quantification of staining was performed by incubating cells in a leaching solution containing 10% acetic acid and 20% methanol to extract the calcium-chelated alizarin red stain. After stain leaching was complete by visual inspection, absorption of samples was measured at 450 nm using an Ultraspec 2100 Pro spectrophotometer (Biochrom Ltd., Cambridge, England). Staining of all samples was performed in triplicate, and all measurements were normalized to the total protein content of a sister well seeded at equal density.

Osteogenic Gene Expression Analysis

The expression profile of early (*RUNX2/CBFA1*) and late (osteocalcin) markers of osteogenic differentiation was assessed using quantitative real-time polymerase chain reaction. RNA was harvested from both suction-assisted lipoaspiration and third-generation, ultrasound-assisted lipoaspiration, adipose-derived stromal cells following 3 days and 10 days of osteogenic differentiation. *RUNX2/CBFA1* expression was evaluated following 3 days of osteogenic differentiation; that of osteocalcin, following 10 days of osteogenic differentiation. Total RNA was isolated using the RNeasy Mini Kit (Qiagen, Valencia, Calif.) according to the manufacturer's protocol for RNA isolation from animal cells. Total RNA was treated with DNAse for 30 minutes at 37°C to remove any contaminating genomic DNA. Reverse transcription of the isolated mRNA was performed using oligo-dT primers and Multiscribe reverse transcriptase (Applied Biosystems, Foster City, Calif.). As a control for genomic DNA contamination, one reaction without reverse transcriptase was also performed, in which 1 μg of DNAse-treated RNA was pooled from all samples equally. The inability to amplify for glyceraldehydes-3-phosphate dehydrogenase from this reaction during polymerase chain reaction confirmed elimination of any contaminating genomic DNA. After reverse transcription, quantitative real-time polymerase chain reaction with SYBR Green (Invitrogen) detection was performed to determine relative expression levels for early (*RUNX2/CBFA1*) and late (osteocalcin) markers of osteogenic differentiation. Quantitative real-time polymerase chain reactions were run in triplicate on 384-well plates and normalized to expression levels of β-actin. Gene expression was analyzed using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems). Published *RUNX2/CBFA1* and osteocalcin quantitative real-time polymerase chain reaction primer sequences were used.[27] β-Actin quantitative real-time polymerase chain reaction primers were obtained from Applied Biosystems (part no. 401846, proprietary sequence).

Statistical Analysis

Given the experimental design, blinded, paired (both samples are derived from a single patient), prospective, and assuming an α value (type I error probability for a two-sided test) of 0.05 and a power (probability of correctly rejecting the null hypothesis) of 0.95, three patients were needed per assay to detect a true difference of 0.20 or greater in population means. These studies were analyzed by using a *t* test (Excel; Microsoft Corp., Redmond, Wash.). The primary outcomes of our investigation were the in vitro proliferative capacity and osteogenic potential of adipose-derived stromal cells as measured by cell counting, alkaline phosphatase activity, extracellular matrix mineralization, and osteogenic gene expression. Given the Poisson distribution of proliferation data, analysis was performed by subjecting the square root of cells counts to a *t* test.

RESULTS

Adipose-derived stromal cells harvested by means of suction-assisted lipoaspiration and third-generation ultrasound-assisted lipoaspiration demonstrate proliferative capacity in vitro that is not significantly different. To begin, proliferative capacity of paired adipose-derived stromal cell samples was compared. Cell counting was performed at 1 day, 3 days, and 7 days after seeding. This window of time was chosen to be inclusive of the period when adipose-derived stromal cells undergo rapid expansion in culture. Nonviable cells were eliminated by staining with trypan blue, and the proliferative capacity of each sample was evaluated in triplicate. The rate of proliferation increased exponentially over the course of the 7-day period of observation, with proliferation being greatest from 3 to 7 days after seeding. At no point during the week-long evaluation of proliferative capacity were significant differences between suction-assisted lipoaspiration– and third-generation ultrasound-assisted lipoaspiration–derived adipose-derived stromal cells observed ($n = 3$) (Fig. 1).

In vitro osteogenic differentiation of adipose-derived stromal cells obtained by means of suction-assisted lipoaspiration and third-generation ultrasound-assisted lipoaspiration is not significantly different. Subsequently, an evaluation was undertaken to determine whether paired adipose-derived stromal cell samples were equipotent in their ability to undergo osteogenic differentiation. After 3 days of culture in osteogenic differentiation media, substantial alkaline phosphatase staining was observed. However, in both adipose-derived stromal cell populations, no gross or microscopic differences in alkaline phosphatase staining were appreciated ($n = 3$) (Fig. 2). After 10 days of osteogenic differentiation of paired adipose-derived stromal cell samples, significant alizarin red staining of calcified extracellular matrix was appreciated in both groups. After stain quantification and normalization of staining to protein content, no significant differences in extracellular matrix mineralization were identified ($n = 3$) (Fig. 3. Of note, control wells with adipose-derived stromal cells cultured in standard growth media in parallel for similar periods did not demonstrate appreciable alkaline phosphatase or aliza-

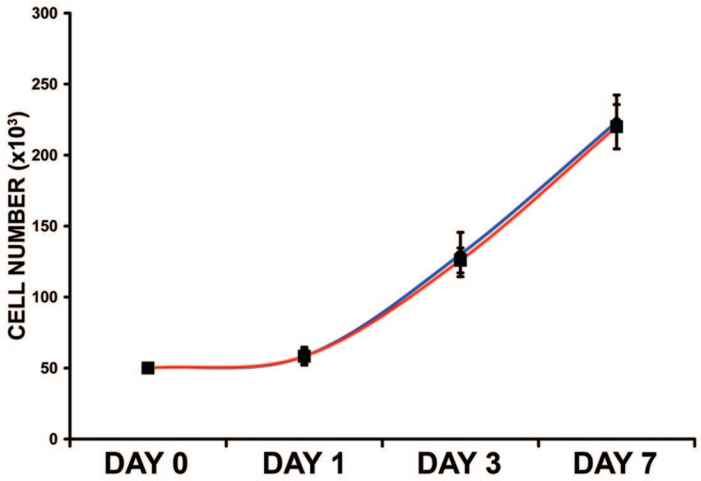

Fig. 1. Proliferation of suction-assisted lipoaspiration– (*red*) and third-generation ultrasound-assisted lipoaspiration–derived (*blue*) adipose-derived stromal cells over a 7-day time course. Findings demonstrate no significant differences in proliferative capacity ($n = 3$). Data are presented as sample mean, and error bars represent SD.

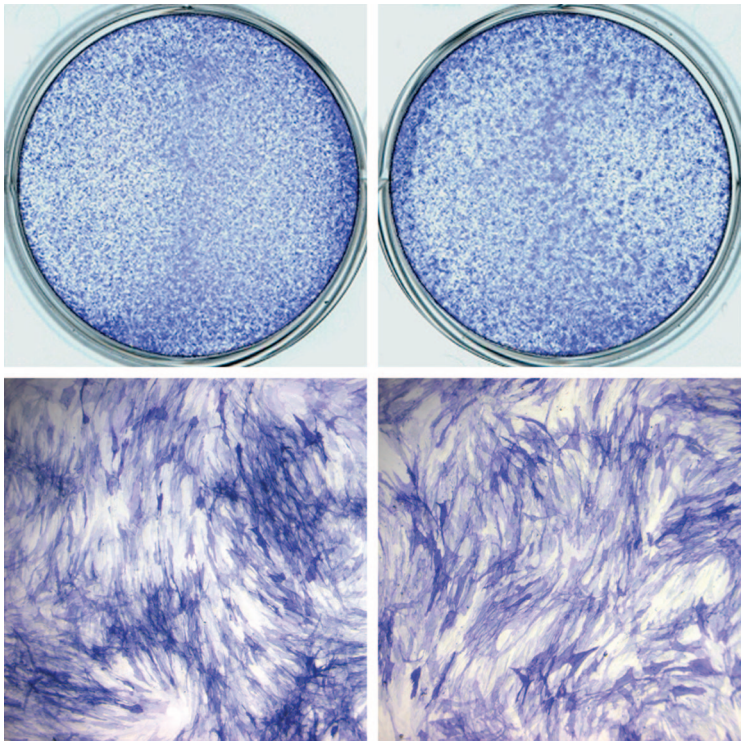

Fig. 2. Alkaline phosphatase staining of suction-assisted lipoaspiration–derived (*left*) and third-generation ultrasound-assisted lipoaspiration–derived (*right*) adipose-derived stromal cells after 3 days of osteogenic differentiation. There were no gross (*above*) or microscopic (*below*, 5× magnification) differences in early osteogenic differentiation.

rin red staining (data not shown). In addition, given the observed similar capacity for suction-assisted lipoaspiration– and third-generation ultrasound-assisted lipoaspiration–derived adipose-derived stromal cells to undergo osteogenic differentiation, a control for our experimental osteogenic differentiation methods was needed to clarify that alternate osteogenic progenitor cell populations possess a differential capacity for in vitro osteogenesis under similar experimental conditions relative to either adipose-derived stromal cell study group. Commercially available bone marrow mesenchymal stem cells (All-Cells), subjected to similar experimental osteogenic differentiation conditions, demonstrated significantly less staining of calcified extracellular matrix (terminal osteogenic differentiation) compared with either suction-assisted lipoaspiration– or third-generation ultrasound-assisted lipoaspiration–derived adipose-derived stromal cells by means of quantified alizarin red staining performed in a similar fashion as for adipose-derived stromal cells (data not shown). Gene expression of markers of early and terminal osteogenic differentiation in adipose-derived stromal cells obtained by means of suction-assisted lipoaspiration and third-generation ultrasound-assisted lipoaspiration is not significantly different.

Finally, quantitative real-time polymerase chain reaction was performed to evaluate gene transcript levels of *RUNX2/CBFA1* and osteocalcin, up-regulated during the processes of early and late osteogenic differentiation, respectively. Quantitative real-time polymerase chain reaction was performed on all samples in triplicate, and transcript levels of genes of interest were normalized to β-actin expression. After 3 days of osteogenic differentiation, *RUNX2/CBFA1* expression was similar in paired adipose-derived stromal cell samples (Fig. 4, *above*). Moreover, no significant differences in osteocalcin expression at terminal differentiation were observed (Fig. 4, *below*).

DISCUSSION

The need for superior skeletal reconstructive modalities is clear. Congruent with this need, evolving knowledge regarding the osteogenic potential of adipose-derived stromal cells is beginning to shed light on the potential role this cell population may play in the future of bone tissue engineering. Sig-

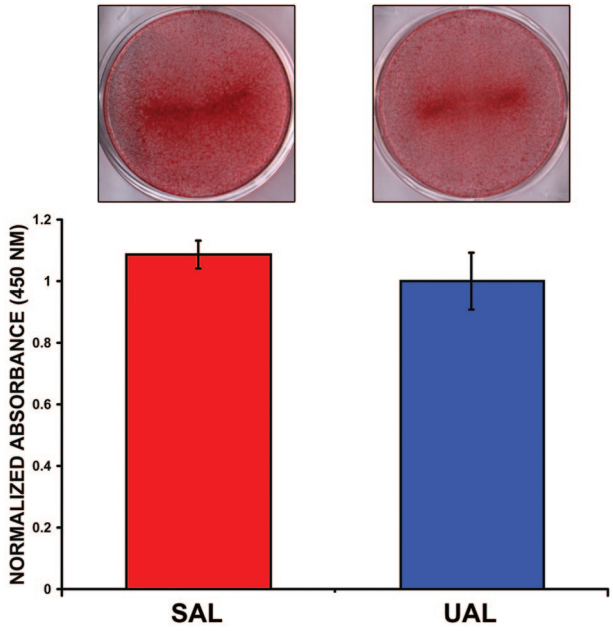

Fig. 3. Alizarin red staining of suction-assisted lipoaspiration (*SAL*)–derived (*red*) and third-generation ultrasound-assisted lipoaspiration (*UAL*)–derived (*blue*) adipose-derived stromal cells after 10 days of osteogenic differentiation. There were no gross differences in extracellular matrix mineralization between the two groups (*above*, $n = 3$). Normalized staining quantification of mineralized extracellular matrix demonstrates no significant difference in terminal osteogenic differentiation (*below*, $n = 3$). Data are presented as sample mean, and error bars represent SD.

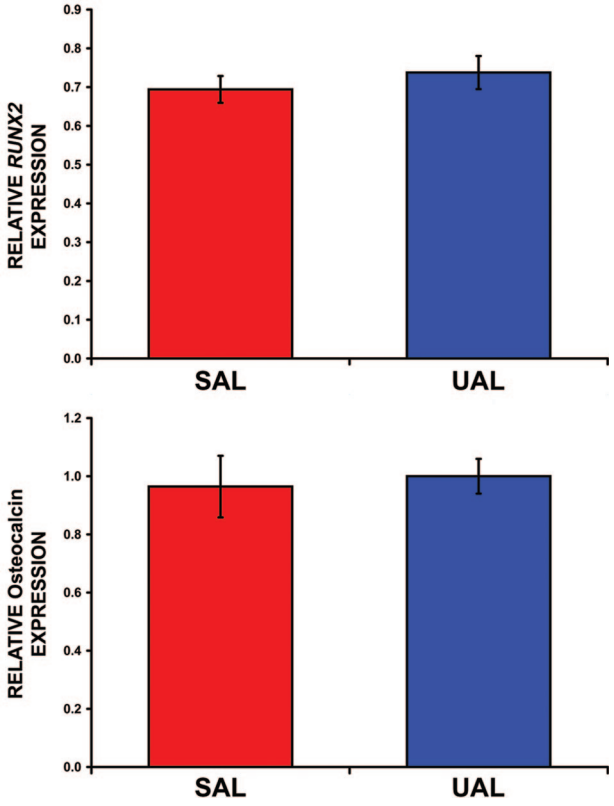

Fig. 4. Relative mRNA levels of *RUNX2* and osteocalcin in suction-assisted lipoaspiration (*SAL*)–derived (*red*) and third-generation ultrasound-assisted lipoaspiration (*UAL*)–derived (*blue*) adipose-derived stromal cells undergoing osteogenic differentiation. No significant differences in transcript levels of *RUNX2* after 3 days of differentiation (*above*, $n = 3$), or of osteocalcin after 10 days of differentiation (*below*, $n = 3$), are observed. Data are presented as sample mean, and error bars represent SD.

nificant progress has been made toward dissecting the molecular biology that underlies the osteogenic potential of adipose-derived stromal cells. It was the work of Zuk and colleagues that first elucidated the in vitro osteogenic potential of this cell population.[1,8] They were able to clearly demonstrate that adipose-derived stromal cells exhibited the potential to calcify extracellular matrix, and elaborate transcripts intimately associated with the stages of early, intermediate, and terminal osteogenic differentiation, when placed in appropriate differentiation conditions.[1,8] Subsequently, a significant body of knowledge in murine animal models has accumulated, extending our knowledge of both the molecular mechanisms driving this lineage-specific differentiation of adipose-derived stromal cells and their potential to heal critical-sized calvarial defects through de novo bone formation.[2,28–30] Such findings lend promise toward the goal of developing translational therapies, and information gleaned from the study of mouse adipose-derived stromal cells has engendered enthusiasm to define whether human adipose-derived stromal cells possess an equal in vitro and in vivo potential to undergo osteogenic differentiation.

Ongoing studies centered on answering these questions are producing compelling evidence that human adipose-derived stromal cells are capable of undergoing potentially even more rapid and robust in vitro osteogenesis than their murine counterparts. Furthermore, there is evidence that the osteogenic potential of human adipose-derived stromal cells is retained in vivo as well. Hicok and colleagues demonstrated that when hydroxyapatite/tricalcium phosphate scaffolds were seeded with human adipose-derived stromal cells and implanted subcutaneously in severe combined immunodeficiency mice, 80 percent of the scaffolds formed osteoid-containing human cells, whereas scaffolds not seeded with adipose-derived stromal cells before implantation demonstrated no osteoid formation.[31] Studies by Yoon et al. lent further support to the in vivo osteogenic potential of human adipose-derived stromal cells. They observed that, when cultured

appropriately before implantation, human adipose-derived stromal cells seeded onto osteoconductive scaffolds and implanted into critical calvarial defects of nude rats produced up to 72 percent regeneration of critical calvarial defects following 12 weeks of healing.[32] This healing was significantly greater than that observed in control groups treated with unseeded scaffolds.[32] Finally, successful endogenous bone regeneration using adipose-derived stromal cells in a human was described by Lendeckel and colleagues. Here, autologous adipose-derived stromal cells, combined with autologous cancellous bone chips, were applied to multiple chronic posttraumatic calvarial defects in a 7-year-old girl, resulting in near complete calvarial healing.[33] Such satisfactory findings continue to stimulate efforts to expand the breadth and depth of studies probing the osteogenic potential of human adipose-derived stromal cells.

This expansion of interest in the study of human adipose-derived stromal cells, coupled with a rising trend in plastic surgery to use third-generation ultrasound-assisted lipoaspiration, have consequently increased the numbers of ultrasound-assisted lipoaspiration-derived adipose specimens that are being made available for the study of bone tissue engineering. Ultrasound-assisted lipoaspiration fundamentally differs from traditional suction-assisted lipoaspiration in that it uses the application of ultrasound energy to selectively emulsify subcutaneous adipose tissue through micromechanical, thermal, and microcavitation effects.[14] Furthermore, significant differences exist in the equipment and energy delivery of varying generations of ultrasound-assisted lipoaspiration modalities, including amplitude setting, probe design, vibration frequency, and mode of energy delivery.[25] First-generation devices delivered ultrasound energy by means of a solid probe in a continuous fashion at a frequency of 20 kHz.[17,25] Second-generation devices used hollow cannulas, allowing for simultaneous ultrasound emulsification and adipose aspiration, and operated at frequencies ranging from 22.5 to 27 kHz.[17,25] However, the continuous delivery of energy at these levels led to excessive complications and morbidity.[17] With the advent of third-generation ultrasound-assisted lipoaspiration devices, probes designs that were smaller in size and grooved were introduced, allowing for energy to be focused at the tip of the instrument. Furthermore, function was modified to deliver pulsed rather than continuous energy.[17] These modifications significantly reduced the energy delivered to treated tissues while maintaining adequate vibration amplitude necessary for the fragmentation of adipocytes.[17,25]

With the realization of technical improvements in ultrasound-assisted lipoaspiration leading to increased clinical implementation, we hypothesized that no significant differences existed in the osteogenic potential of human adipose-derived stromal cells harvested by means of suction-assisted lipoaspiration or third-generation ultrasound-assisted lipoaspiration. The importance of investigating this hypothesis cannot be overstated, as a significant decrease in osteogenic potential resulting from exposure to ultrasound energy during the course of lipoaspiration using a third-generation device would deter their use in the study of skeletal tissue engineering and their application in the development of translational therapies. As such, we are very encouraged by the observed absence of significant differences in the in vitro osteogenic potential of suction-assisted lipoaspiration–derived and third-generation ultrasound-assisted lipoaspiration–derived adipose-derived stromal cells.

CONCLUSIONS

Our data demonstrate that exposure to ultrasound energy by means of third-generation ultrasound-assisted lipoaspiration does not impair the in vitro osteogenic potential of human adipose-derived stromal cells relative to adipose-derived stromal cells obtained by means of suction-assisted lipoaspiration. The absence of significant differences in the in vitro proliferative capacity, potential for osteogenic differentiation, and osteogenic gene transcript expression levels in suction-assisted lipoaspiration–derived and third-generation ultrasound-assisted lipoaspiration–derived adipose-derived stromal cells puts forth that the harvest of adipose-derived stromal cells using ultrasound energy imparts no effects on the adipose-derived stromal cells that would discourage their future use in the study of bone tissue engineering. It will be the goal of future studies to extend these findings and elucidate whether the potential of adipose-derived stromal cells exposed to ultrasound energy during harvest maintain their osteogenic potential in vivo and their potential to differentiate toward alternative mesenchymal lineages, including adipogenic, myogenic, and chondrogenic differentiation, in vitro and in vivo.

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