Adipose-Derived Stem Cells Combined with a Demineralized Cancellous Bone Substrate for Bone Regeneration

Yaling Shi, Ph.D.,1 Jerry R. Niedzinski, B.S.,2 Adrian Samaniego, B.S.,1 Simon Bogdansky, Ph.D.,1 and Brent L. Atkinson, Ph.D.1

Mesenchymal stem cells (MSCs) isolated from cadaveric adipose tissue can be obtained in large quantities, and have been reported in the literature to be capable of inducing bone formation in vivo and ex vivo.1–6 The hypothesis tested whether a demineralized cancellous bone matrix (DCBM) can provide an effective substrate for selection and retention of stem cells derived from the stromal vascular fraction (SVF) of adipose. Human cadaveric adipose tissue was recovered from a donor and digested. The resulting SVF-containing MSCs were seeded onto the demineralized bone allografts, after which the nonadherent cells were washed off. The MSCs were characterized using a flow cytometer and tri-lineage differentiation (osteogenesis, chondrogenesis, and adipogenesis) in vitro. The stem cell-seeded allografts were also characterized for cell number, adherence to the DCBM, osteogenic activity (alkaline phosphatase and Alizarin Red staining), and bone morphogenetic protein (BMP) quantity. Flow cytometry identified a mean total of 7.2% MSCs in SVF and 87.2% MSCs after culture. The stem cells showed the capability of differentiating into bone, cartilage, and fat. On the 21 stem cell-seeded bone allografts, there were consistent, attached, viable cells (100,744–22,762 cells/cube). An assessment of donor age, gender, and body mass index revealed no significant differences in cell numbers. Enzyme-linked immunosorbent assay revealed the presence of BMP-2 and BMP-7. In conclusion, this bone graft contains three key elements for bone regeneration: adhered osteogenic stem cells, 3D osteoconductive bone scaffold, and osteoinductive BMP signal. It therefore has the potential to be effective for bone regeneration.

Introduction

Mesenchymal stem cells (MSCs) reside in many tissues, including bone marrow, adipose tissue, synovial fluid, dermis, and muscle.7 MSCs can differentiate along a variety of cell lineages that can be used to regenerate bone and other tissues. Because adipose tissue is the most abundant and accessible source of adult stem cells, it thus has great potential for tissue regeneration. Adipose-derived MSCs share many of the characteristics of bone marrow-derived stem cells (BMSCs), including extensive proliferative potential, but are much more abundant and easier to recover with a higher proliferation rate than BMSCs. They are also remarkably similar with respect to morphology, displaying fibroblastic characteristics, with abundant endoplasmic reticulum and a large nucleus relative to the cytoplasmic volume. Other common characteristics can be found in the transcriptional and cell surface profile.8–11 MSCs display extensive self-renewal capacity to undergo differentiation into many mesenchymal cell types. Moreover, MSCs have been reported to have low immunogenicity.12,13 Many studies have reported bone regeneration using MSCs from adipose tissue.2,6,14–18 These studies have demonstrated that stem cells obtained from adipose tissue are capable of exhibiting good attachment properties to most material surfaces and have the capacity to differentiate into osteoblast-like cells in vitro and in vivo. Recently, it has been shown that adipose-derived stem cells (ASCs) may stimulate the vascularization process.19,20

Demineralized cortical bone matrix (DBM), as an allogeneic material, is a well-utilized bone tissue-engineering scaffold due to its close relation to autologous bone in terms of composition, structure, and function. Derived from native osseous tissue, demineralized bone contains bone morphogenetic proteins (BMPs) and matrix proteins. BMPs are potent osteoinductive glycoproteins, whereas matrix proteins, such as different collagens, provide an osteoconductive matrix. Combined with MSCs, these scaffolds have been
demonstrated to accelerate and enhance bone formation within osseous defects when compared with the matrix alone.\textsuperscript{3,21–31} Stem cell-seeded bone allografts therefore have great potential for bone regeneration.

The current experiment was designed to test the hypothesis that demineralized cancellous bone matrix (DCBM) provide an effective alternative substrate for selection and retention of stem cells derived from the stromal vascular fraction (SVF) of adipose tissue (nonculture expanded). The objective of this study was to seed adipose-derived SVF onto DCBM and characterize these stem cell-seeded grafts for differentiation potential, cell phenotype, cell number, and donor variability, adherence to DCBM, \textit{in vitro} osteogenic activity, and BMP quantity with respect to the utility for bone repair.

Materials and Methods

\textbf{Isolation of MSCs from human adipose tissue}

Human cadaveric adipose tissue was recovered within 24 h of a donor’s death and shipped in an equal volume of the Dulbecco’s modified Eagle’s medium (DMEM) in wet ice to AlloSource. Adipose tissue was washed and digested with Collagenase Type I. Subsequently, it was centrifuged at 500 g at room temperature. The supernatant, containing mature adipocytes, was aspirated. The pellet was identified as the SVF. The cells were counted using an automatic cell counter (Countess Cell Counter, Invitrogen) and plated onto a culture flask at a density of 300,000/cm\textsuperscript{2} to establish the primary culture.

\textbf{Demineralization of bone grafts}

Human cancellous bone was recovered from the same donor and sectioned into cubes (10×10×10 mm). The grafts were then placed in a demineralization solution and rinsed until the pH was neutral. The grafts were then stored in a growth medium (DMEM/F12+10% fetal bovine serum [FBS]).

\textbf{Stem cell-seeded bone allograft}

The demineralized allograft cubes were combined with the SVF at a concentration of 1 million cells from SVF per cube. Then, the grafts were incubated at 37°C with 5% CO\textsubscript{2} and 100% humidity and rinsed thoroughly in phosphate-buffered saline (PBS) to remove nonadherent cells. Subsequently, the allografts were placed into cryopreservation media (10% dimethyl sulfoxide, 90% PBS) and frozen at --80°C. The donor age, gender, and body mass index (BMI) from 21 donors were recorded for cell counting.

\textbf{MSC characterization}

\textbf{Flow cytometry analysis.} The following antibodies were used for flow cytometry: PE anti-CD73 (clone AD2), FITC anti-CD34 (clone 8G12), FITC anti-CD45 (clone 2D1); the isotype controls, FITC Mouse IgG1 Kappa (clone MOPC-21), PE Mouse IgG1 Kappa (clone MOPC-21), and PE Mouse IgG2a Kappa (clone G155-178), were all purchased from Becton Dickinson. PE anti-CD271 (clone ME20.4-1.H4) was purchased from Miltenyi BioTec. PE anti-CD90 (clone F15-42-1), PE anti-CD105 (clone SN6), and PE anti-Fibroblasts/Epithelial Cells (clone D7-FIB) were purchased from AbD SeroTec. Freshly isolated SVF cells and cells cultured up to three passages on tissue culture plastic (P1–P3) were analyzed for surface marker expression. P1–P3 cells were harvested with 2 mM ethylenediaminetetraacetic acid/PBS (pH 1). All of the following steps were performed on ice. A small aliquot of the cells was stained with a propidium iodide/detergent solution, and fluorescent nuclei were counted using a hemocytometer on a fluorescent microscope. This total cell count was used to adjust the number of cells per staining tube to no more than 5.0×10\textsuperscript{5} cells. The cells were washed with a flow cytometric wash buffer (PBS supplemented with 2% FBS and 0.1% Na\textsubscript{3}N\textsubscript{3}) stained with the indicated antibodies, and washed again before acquisition. Staining was for 15 min at room temperature (15°C–30°C). At least 20,000 cells were acquired for each sample on a FACScan flow cytometer equipped with a 15-mW, 488-nm, and argon-ion laser (BD Immunocytometry Systems). The cytometer QC and setup included running Spherotech rainbow (3 μm, 6 peaks) calibration beads (Spherotech Inc.) to confirm instrument functionality and linearity. Flow cytometric data were collected and analyzed using CellQuest software (BD Immunocytometry Systems). The small and large cells were identified by forward (FSC) and side-angle light scatter (SSC) characteristics. Autofluorescence was assessed by acquiring cells on the flow cytometer without incubating with fluoro-chrome-labeled antibodies. Surface antigen expression was determined with a variety of directly labeled antibodies according to the supplier’s recommendations. The viability of the small and large cells was determined using the Becton Dickinson Via-Probe (7-AAD).

\textbf{In-vitro tri-lineage differentiation}. 100,000 MSCs at passage 2 in 5 mL were plated into 18-wells on three 6-well plates that contained the growth medium. The growth medium was changed every 3 to 4 days until the cells reached 70% confluence.

\textbf{Osteogenesis.} MSCs in three wells were induced to undergo osteogenesis by replacing the growth medium with an osteogenic induction medium (Stempro® osteogenesis differentiation kit; Invitrogen). The growth medium was replaced with a fresh osteogenic induction medium, and the latter medium was replaced every 3 to 4 days for a period of up to 3 weeks. The other three wells on the same plate were cultured with a growth medium for up to 3 weeks as non-differentiated controls. Cells were then fixed in 10% neutral buffered formalin and rinsed with deionized water. Osteogenic differentiation was determined by staining for calcium phosphate with Alizarin red (Sigma).

\textbf{Adipogenesis.} MSCs in three wells were induced to undergo adipogenesis by replacing the growth medium with an adipogenic induction medium (Stempro adipogenesis differentiation kit; Invitrogen). The growth medium was replaced with a fresh adipogenic induction medium, and the latter medium was replaced every 3 to 4 days for a period of up to 2 weeks. The other three wells on the same plate were cultured with a growth medium up to 2 weeks as non-differentiated controls. Cells were then fixed in 10% neutral buffered formalin and rinsed with PBS. Adipogenic differentiation was determined by staining for fat globules with Oil red O (Sigma).
Chondrogenesis. Confluent cultures of primary MSCs were induced to undergo chondrogenesis by replacing the growth medium with a chondrogenic induction medium (Stempro chondrogenesis differentiation kit; Invitrogen). The growth medium was replaced with a fresh chondrogenic induction medium, and the latter medium was replaced every 3 to 4 days for a period of up to 4 weeks. The other three wells were cultured with a growth medium up to 4 weeks as nondifferentiated controls. Cells were then fixed in 10% neutral buffered formalin and rinsed with PBS. Chondrogenic differentiation was determined by staining for proteoglycans with Alcian blue (Sigma).

Stem cell-seeded allograft characterization

Histology. The cryopreserved stem cell-seeded bone graft samples were thawed and rinsed with PBS, then fixed in 10% neutral buffered formalin (Sigma) for 48 h, placed in a tissue processor (Citadel 2000; Thermo Shandon) overnight, and embedded in paraffin. Sections were cut to 5-µm thickness, mounted onto glass slides, and stained with hematoxylin and eosin (H&E). Conventional light microscopy was used to analyze sections for matrix and cell morphology.

Scanning electron microscopy. The cryopreserved stem cell-seeded and nonseeded samples from the same donor were thawed, rinsed, and then fixed in 4% paraformaldehyde-phosphate buffer (Wako Pure Chemical Industries, Ltd.) and 2% osmic acid (Wako Pure Chemical). After dehydration through graded series of water-ethanol and ethanolisopropanol acetate solutions, the samples were dried by a lyophilizer (HCP-2; Hitachi Koki Co., Ltd.). Samples were subsequently shadowed with gold using an ion sputter (JFC100) and observed with a scanning electron microscope (JSM-840; Nihon Denshi Corp.).

Cell count: CCK-8 assay. Cell Counting Kit 8 (CCK-8; Dojindo Molecular Technologies) allows sensitive colorimetric assays for the determination of viable cell quantity. WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt] is reduced by dehydrogenases in cells to produce a yellow-colored product (formazan), which is soluble in the tissue culture medium. The amount of the formazan dye generated by the activity of dehydrogenases in cells is directly proportional to the number of living cells. The stem cell-seeded bone grafts were thawed and rinsed with PBS and then patted dry. The growth medium and CCK-8 solution were added into the allografts at a ratio of 10:1, cultured at 37°C for 2 h, and evaluated in a plate reader with an excitation wavelength set to 460 nm and emission wavelength set to 650 nm. The results were interpolated from a standard curve based on ASCs only (passage = 3).

Table 1. Immunophenotype of the Stromal Vascular Fraction (n=5)

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<thead>
<tr>
<th></th>
<th>% of Total</th>
<th>Viability</th>
<th>D7-FIB</th>
<th>CD105</th>
<th>CD90</th>
<th>CD73</th>
<th>CD271</th>
<th>CD34</th>
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Bone morphogenetic proteins. The method based upon that of Blum et al. was used in this study to extract and quantify BMP-2, BMP-4, and BMP-7. Briefly, lyophilized demineralized allograft cancellous bone cubes (n = 9) were rehydrated in 5 mL collagenase reconstitution buffer for 22 h in a 37°C water bath with constant agitation (130–150 rpm). At the end of the incubation period, the digested samples were centrifuged for 20 min at 1000–1300 g. The supernatants were transferred to a clean 15-mL conical tube, which was placed on wet ice. The enzyme-linked immunosassay (ELISA; R&D Systems) was performed to quantify BMP-2, 4, and 7. Stem cell-seeded demineralized cancellous bone cubes from 3 different donors (3 cubes per donor, n = 9 in total) in a cryopreservation medium were tested.

Statistical analysis

All quantitative data were expressed as the mean ± standard deviation. Statistical analysis was performed with the one-way analysis of variance with Microsoft Excel. A value of p ≤ 0.05 was considered statistically significant.
Results

MSC characterization

Immunophenotype of SVF and ASCs. The SVF was stained with CD105, CD90, and CD73 to determine if there were significant numbers of MSCs present. The SVF had both large and small cells (Table 1). The SVF contained a significant population of CD34+ cells. The paucity of CD45+ cells suggested that the SVF did not contain significant numbers of WBCs or hematopoietic stem cells. The immunophenotype of the SVF was consistent from donor to donor.

Following adherence of the SVF to tissue culture flasks (MSCs, Passage 1), the immunophenotype became more homogenous for both the large and small cells (Table 2). After further culturing (P3), the ASCs lost CD34 marker expression, and the CD45+ cells remained low. The anti-Fibroblasts/Epithelial Cell (D7-FIB) antibody showed an increased expression.

CD105 was chosen to estimate the mean total percentage of MSCs. Combining the large and small cells resulted in a mean total of 7.2% MSCs for the SVF. For the adherent and cultured SVF (P1), combining the large and small cells gave a total of 87.2% MSCs.

In vitro tri-lineage differentiation. MSCs cultured in osteogenic, adipogenic, and chondrogenic induction media were able to differentiate into any of the three lineages. For osteogenic differentiation, morphological changes appeared during the second week of the culture. At the end of the 21-day induction period, mineral deposition was confirmed by Alizarin Red staining (Fig. 1A). The adipogenic potential was assessed by induction of adipogenesis of confluent ASCs. At the end of the induction cycles (7 to 14 days), a consistent cell vacuolation was evident in the induced cells. Vacuoles brightly stained for fatty acid with Oil Red O staining (Fig. 1B). Chondrogenic potential was assessed by chondrogenic induction of confluent ASCs. At the end of the induction cycles (21 to 28 days), the induced cells were morphologically different from noninduced control cells. Cell differentiation was confirmed with Alcian Blue staining, an indicator of glycosaminoglycan formation, a distinguishing component for cartilage (Fig. 1C).

Stem cell-seeded allograft characterization

Histology. H&E was performed to demonstrate cell morphology in relation to the underlying substrate (cancellous bone matrix). After 36 h of incubation, the cells were elongated and adhered to the surface of cancellous bone (Fig. 2).

![Fig. 1.](https://www.liebertonline.com/tea)

**FIG. 1.** Mineral deposition by ASCs cultured in an osteogenic medium (A), indicating early stages of bone formation. The samples were stained with Alizarin red S. Negative controls (D) showed no sign of bone formation. Fat globules seen in ASCs cultured in an adipogenic medium (B), indicating differentiation into adipocytes. The samples were stained with Oil red O. The picture E is the negative control. Proteoglycans produced by ASCs cultured in a chondrogenic medium (C), indicating early stages of chondrogenesis. The samples were stained with Alcian Blue. The negative control (F) showed no sign of chondrogenesis. Color images available online at www.liebertonline.com/tea

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Note that all surface antigens tested are consistent with mesenchymal stem cells.

Table 2. Immunophenotype of Adherent Cells
Scanning electron microscopy. Scanning electron microscopy (SEM) revealed the difference of allograft cancellous bone with and without cells at the microstructural level. Figure 3A showed the typical 3D microporous structure of demineralized cancellous bone matrix without any cells. Figure 3B revealed that the cells were attached to the cancellous matrix and stayed alive.

Cell counts: CCK-8 Assay. There were 98,336 \( \pm \) 30,402 cells/cube \((n = 11)\) from donors whose age was 45 (included) to 55. There were 103,393 \( \pm \) 10,465 cells/cube \((n = 10)\) from donors whose age was 55 (included) to 65 (Fig. 4A). There was no significant difference regarding the cell number on cubes from the two different donor age groups.

There were 99,433 \( \pm \) 12,493 cells/cube \((n = 16)\) from male donors (Fig. 4B). There were 104,940 \( \pm \) 12,493 cells/cube \((n = 5)\) from female donors. There was no significant difference regarding the cell number on cubes.

There were 92,430 \( \pm \) 7,463 cells/cube \((n = 3)\) from donors whose BMI (19 to 24) was normal. There were 107,746 \( \pm \) 18,579 cells/cube \((n = 3)\) from donors whose BMI (25 to 29) was overweight. There were 99,827 \( \pm \) 13,670 cells/cube \((n = 7)\) from donors whose BMI (30 to 39) was obese. There were 94,737 \( \pm \) 70,294 cells/cube \((n = 6)\) from donors whose BMI (40 to 54) was extremely obese. There were 131,788 \( \pm \) 70,294 cells/cube \((n = 2)\) from donors whose BMI (> 54) was beyond extremely obese (Fig. 4C). There was no significant difference regarding the cell number on cubes from the five different donor BMI groups.

In vitro osteogenic differentiation. The ALP activity of the allografts with cells was 14.3 \( \pm \) 1.1 \(\mu\)mol/mL per cube. There was 5.5 \(nmol\) of ALP/mL/\(10^6\) cells generated in vitro after normalized to cell numbers from stem cell-seeded allografts. In addition, the allografts showed mineral deposits stained with Alizarin Red (Fig. 5).

Bone morphogenic proteins. There was 3706.3 \( \pm \) 3416.8 pg of BMP-2/g dry cube and 42871 \( \pm \) 24965.8 pg of BMP-7/g dry cube, respectively (Fig. 6). BMP-4 was only detected in 2 cubes.

Discussion

The concept of having adipose-derived MSCs seeded onto allogeneic bone has been pursued by many researchers. However, the biggest difference between this study and all other published references1,4,14,17,21,23,26,27,33–42 is that the SVF was used directly to seed onto demineralized bone, and all other studies used cultured MSCs. The current experiment was designed to test the hypothesis that demineralized cancellous bone matrix (DCBM) provide an effective alternative substrate for selection and retention of stem cells derived from the SVF of adipose tissue. The objective of this study was to seed the adipose-derived SVF onto the DCBM and characterize these stem cell-seeded grafts for differentiation potential, cell phenotype, cell number, donor variability, adherence to DCBM, osteogenic activity (ALP), and BMP quantity with respect to the utility for bone repair. Our study has successfully demonstrated that the SVF from adipose can be used directly to seed MSCs onto demineralized bone grafts from the same donor.
The ASCs described herein showed all of the characteristics of MSCs as reported by the International Society for Cellular Therapy (ISCT), which included tri-lineage differentiation, adherence, and immunophenotype (CD105, CD90, and CD73 positive; CD45 negative). Multiple independent groups have examined the surface immunophenotype of ASCs isolated from human and other species. Despite any differences in the isolation and culture procedures, the immunophenotype is relatively consistent among published reports for both the SVF and cultured MSCs, and the data from this study are consistent with those reports. Furthermore, the surface immunophenotype of ASCs resembles that of bone marrow-derived MSCs and skeletal muscle-derived cells. Additional studies have demonstrated that the expression profile changes as a function of time in passage and plastic adherence, and our results are also confirmatory.

MSCs or osteoprogenitors are well accepted to play a critical role for bone information. This study has successfully demonstrated that the SVF from adipose can be used directly to seed MSCs onto demineralized bone grafts from the same donor. The resulting stem cell-seeded bone grafts from 21 donors appeared to have attached cells and consistent cell numbers regardless of donor age, gender, and BMI. These

![Image](image1.png)

**FIG. 4.** Cell counts of allografts from different age (A), gender (B), and body mass index (BMI) (C) groups. Note that there was no significant difference regarding the cell number on cubes from different donor age, gender, and BMI groups.

![Image](image2.png)

**FIG. 5.** There were sixfold greater osteogenic activities from allografts with stem cells than those without stem cells. Significant differences were found between these two groups. Color images available online at www.liebertonline.com/tea

![Image](image3.png)

**FIG. 6.** Bone morphogenic protein (BMP) content in the cubes. BMP-2 and BMP-7 were all well detected. BMP-4 was not detected in 7 cubes.
glands also showed the capability for in-vitro osteogenesis. Furthermore, the cancellous bone grafts demonstrated the presence of osteoinductive BMPs.

Since animal and clinical data demonstrate that the number of endogenous or host osteoblastic progenitor cells in many graft sites is suboptimal, there is a need to supplement bone grafts with cells to improve the clinical outcome. A few studies have been performed to assess the quantity of cells required to reach an improved outcome. One research group used bone marrow aspirate combined with demineralized cortical bone powder in an established canine spinal fusion model.54 Fusions were compared based on the union score, fusion mass, fusion volume, and by mechanical testing. Enriched matrix grafts delivered a mean of 2.3 times more cells and approximately 5.6 times more progenitors than the matrix mixed without bone marrow. They showed that the mean number of connective tissue progenitors implanted with the graft required for successful spinal fusion was 39,400 ±24,500. In contrast, in unsuccessful fusions, ∼5× fewer connective tissue progenitors were present. Fusion volume and fusion area data supported that treatments with the higher number of connective tissue progenitors improved bone formation. Another study has shown that bone union was obtained with an average total of 54,962 ±17,431 progenitors from concentrated bone marrow aspirates injected into tibial fracture nonunion sites in 53 patients. The bone union was not obtained with an average total of 19,324 ±6843 progenitors injected into the nonunion sites of seven patients. Each nonunion received a relatively constant volume of 20 cc of concentrated bone marrow. There was a positive correlation between the volume of mineralized callus at four months and the number \( p=0.04 \) and concentration \( p=0.01 \) of fibroblast colony-forming units in the graft. There was a negative correlation between the time needed to obtain union and the concentration of fibroblast colony-forming units in the graft \( p=0.04 \). Thus, these two studies suggested that there were a minimum number of cells required to promote a successful outcome in two bone fusion studies.

Unlike the variability in cell numbers observed with bone marrow aspirate, this study demonstrated consistent and high cell numbers (100,744 ±22,762) in ASC-seeded bone grafts. The number of cells observed per mL of bone graft was ~36-fold greater than the amount used in the above nonunion cases. Additional studies are required to determine whether ASCs have the same effect clinically as cells derived from bone marrow aspirate.

Demineralized cortical bone grafts have been demonstrated to contain osteoinductive BMPs. These proteins play pivotal roles in bone formation, as well as osteoblast differentiation. There is a significant, positive, linear relationship between the in vivo new bone formation and BMP-2, BMP-4, and BMP-7 and TGF-β1 levels in the human DBM. Researchers have cited BMP-2 levels ranging from 2.11 ±1.26 to 65.8 ±48.5 ng/g DBM (cortical bone only). BMP-4 levels either were undetectable or were measured to be 3.70 ±0.21 ng/g DBM, and there was ~10 times more BMP-7 than BMP-2 in published results. Interestingly, not a lot of research has been published regarding BMPs in the human demineralized cancellous bone matrix, since it is believed that cancellous bone only provides 3D osteoconduct, not osteoinduction. Some researchers stated that the cancellous bone does not have a significant amount of BMPs. Our data showed that the averages of BMP-2, BMP-4, and BMP-7 (3.71 ±3.42 ng of BMP-2/g dry cancellous cube and 42.87 ±24.96 ng of BMP-7/g) in stem cell-seeded cancellous allografts were all in the range of those published for the cortical-derived DBM. Thus, these cancellous bone grafts seeded with stem cells appear to provide osteoinductive signals and may help to promote osteoinduction in vivo.

Combined with MSCs, these demineralized bone scaffolds have been demonstrated to accelerate and enhance bone formation within osseous defects when compared with the matrix alone.22–32 The ability of the DBM to enhance osteogenesis of MSCs in vitro and in vivo is believed to be due to the interaction of osteoprogenitors with these matrix-incorporated osteoinductive factors, which can induce MSCs into osteoblasts. In turn, the incorporation of an osteogenic cell source into the DBM can potentially limit the need for the migration and expansion of indigenous osteoprogenitors within defect sites, allowing for an increased rate of bone formation and osseointegration. Human adipose-derived MSCs offer a unique advantage in contrast to other cell sources. The multipotent characteristics of adipose-derived MSCs, as well as their abundance in the human body, make these cells a popular source in tissue-engineering applications.

In conclusion, this study has successfully demonstrated that ASCs derived from the SVF of adipose have all of the characteristics of MSCs, which include adherence, the presence of CD markers CD105, CD90, and CD73, and the capability of tri-lineage differentiation. Furthermore, the SVF was used to seed demineralized bone grafts from the same donor. The resulting stem cell-seeded bone grafts contained attached stem cells and consistent cell numbers regardless of donor age, gender, and BMI. In conclusion, this bone graft contains three key elements for bone regeneration: adhered osteogenic stem cells, 3D osteoconductive bone scaffold, and osteoinductive BMP signal. It therefore has the potential to be effective for bone regeneration. Clinical testing of these promising candidates for bone repair is ongoing.

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Disclosure Statement

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Address correspondence to:
Yaling Shi, Ph.D.
AlloSource
6278 S. Troy Circle
Centennial, CO 80111
E-mail: yshi@allosource.org
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