TISSUE ENGINEERING
Volume 11, Number 3/4, 2005

Engineered Adipose Tissue from Human Mesenchymal Stem Cells Maintains Predefined Shape and Dimension: Implications in Soft Tissue Augmentation and Reconstruction

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ABSTRACT

Soft tissue augmentation is a widespread practice in plastic and reconstructive surgery. The objective of the present study was to engineer adipose tissue constructs with predefined shape and dimensions, potentially utilizable in soft tissue augmentation and reconstruction, by encapsulating adult stem cell-derived adipogenic cells in a biocompatible hydrogel system. Bone marrow-derived adult human mesenchymal stem cells (hMSCs) were preconditioned by 1 week of exposure to adipogenic-inducing supplement followed by photoencapsulation in poly(ethylene glycol) diacrylate (PEGDA) hydrogel in predefined shape and dimensions. In two parallel experiments, the resulting hMSC-derived adipogenic cell–polymer constructs were either incubated *in vitro* in adipogenic medium or implanted *in vivo* in the dorsum of immunodeficient mice for 4 weeks. Tissue-engineered adipogenic constructs demonstrated positive reaction to oil red O staining both *in vitro* and *in vivo*, and expressed PPAR-γ2 adipogenic gene marker *in vivo*. By contrast, control PEGDA hydrogel constructs encapsulating undifferentiated hMSCs failed to demonstrate the adipogenic gene marker and were negative for oil red O staining. Recovered *in vitro* and *in vivo* constructs maintained their predefined physical shape and dimensions. These data demonstrate that adipose tissue engineered from human mesenchymal stem cells can retain predefined shape and dimensions for soft tissue augmentation and reconstruction.

INTRODUCTION

PLASTIC AND RECONSTRUCTIVE SURGEONS are constantly burdened with the challenge of replacing lost soft tissue due to trauma, diseases, or congenital anomalies. Mastectomy and tumor resection procedures are merely examples of nonelective surgical procedures that mandate the replacement of lost soft tissue to restore physical shape and/or physiological function.[1–4] More than 6.2 million individuals received reconstructive plastic surgery procedures in 2002, approximately 70% of them as a result of tumor removal.[5] Elective cosmetic procedures also require the placement of soft tissue implants to restore or improve tissue contour to enhance esthetic appearance.[6–9] In 2002, a total of 6.6 million individuals underwent a cosmetic surgery procedure.[5] In the majority of cases of soft tissue augmentation or reconstruction, the missing soft tissue is largely composed of subcutaneous adipose tissue.[2,10,11]

The current standard of care for soft tissue reconstruction and augmentation includes the utilization of synthetic implants or tissue transfer.[12–14] The patient's

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own adipose tissue is preferable to avoid potential complications associated with allografts, xenografts, and synthetic materials such as pathogen transmission and immune rejection.[15–17] Conventional soft tissue-grafting procedures have had certain levels of clinical success for soft tissue augmentation and reconstruction.[8,15–26] However, the need for secondary surgical procedures for autologous tissue harvest and the average of 40–60% reduction in graft volume over time are considered drawbacks of current autologous fat transplantation procedures.[1,2,6,24] By design, tissue-engineered soft tissue grafts from the patient's own adult stem cells should overcome the shortcomings associated with current soft tissue-grafting modalities.[11,12,14,27–29]

Mesenchymal stem cells (MSCs) are capable of differentiating into connective tissue-forming cell lineages such as cartilaginous, osseous, and adipose tissues.[30–32] For adipose tissue engineering, the use of adult MSCs may be advantageous over the use of differentiated adipocytes, because mature adipocytes, although readily attainable from clinical procedures such as lipectomy, have low expandability and poor ability for volume retention following *in vivo* soft tissue reconstruction.[1–3] MSCs, which can be isolated via minimally invasive procedures from the bone marrow or other connective tissue sources of the patient, are highly expandable in culture and can be readily induced to differentiate into adipose tissue-forming cells after exposure to a well-established adipogenic tissue-inducing supplement.[29,31–33]

The next element in a cell-based tissue-engineering approach is the scaffolding material. Successful scaffolding material for adipose tissue engineering must be biocompatible, support tissue formation, and demonstrate considerable stability to allow for successful integration between the newly formed tissue and the surrounding host tissue.[34] An increasing number of biocompatible polymers have been utilized as scaffolds for tissue engineering of bone,[33–39] cartilage,[40–46] and adipose tissue.[2,7,10,47–50] Hydrogels are hydrophilic, three-dimensional networks of polymers that absorb large amounts of water or biological fluids while maintaining their distinct three-dimensional (3-D) structure.[36] Hydrogel scaffolds provide tissue-forming cells with an environment mimicking the extracellular matrix.[36,51] Among the hydrogel family, poly(ethylene glycol)-based hydrogel polymers have certain advantages for several tissue-engineering applications, not only because of their proven biocompatibility,[52–57] but also for their demonstrated capacity to support the growth and differentiation of MSCs into multiple lineages such as bone[32,52–57] and cartilage.[32,56–60] An added advantage of encapsulating adult stem cells in such an injectable hydrogel system is the possibility of transdermal photopolymerization of the hydrogel implant after subcutaneous administration via simple injection,[44] an approach that is especially attractive for adipose tissue engineering.[36,61]

The present study was designed to engineer adipose tissue constructs with predefined shape and dimensions by encapsulating adult stem cell-derived adipogenic cells in a photopolymerizable poly(ethylene glycol) diacrylate (PEGDA) hydrogel system. Complementary *in vitro* and *in vivo* approaches were designed to demonstrate not only the differentiation of bone marrow-derived human mesenchymal stem cells into adipogenic cells, but also the volume stability of tissue-engineered constructs after 4 weeks of *in vitro* incubation and *in vivo* implantation.

MATERIALS AND METHODS

Isolation and culture of human mesenchymal stem cells

Human MSCs (hMSCs) were isolated from whole bone marrow aspirates of the iliac crest of a healthy 22-year-old male donor (AllCells, Berkeley, CA). Briefly, cells were enriched by adding RosetteSep mesenchymal enrichment cocktail (StemCell Technologies, Vancouver, BC, Canada) to the whole bone marrow sample. Sample was then diluted with twice the volume of phosphate-buffered saline (PBS) containing 2% fetal bovine serum (FBS) (Atlanta Biologicals, Lawrenceville, GA) and 1 mM ethylenediamine tetraacetic acid (EDTA) (Sigma, St. Louis, MO). The diluted sample was layered on top of a Ficoll-Paque (StemCell Technologies) cushion and centrifuged for 25 min at $300 \times g$. Enriched cells were removed from the Ficoll-Paque plasma interface and washed with PBS containing 2% FBS and 1 mM EDTA, and cells were resuspended in complete culture medium: MesenCult basal medium for hMSCs (StemCell Technologies), 10% hMSC stimulatory supplements (StemCell Technologies), and 1% antibiotic–antimycotic (GIBCO; Invitrogen, Carlsbad, CA). Cells were counted, plated in 10 mL of complete medium in 100-mm culture dishes, and incubated in 95% air–5% CO_2 at 37°C. After 24 h, nonadherent cells were discarded; adherent cells were washed twice with PBS and incubated for 10–14 days with a fresh medium change every 3–4 days. When large colonies had formed (typically 10–14 days) and before confluence, primary hMSCs were trypsinized with 0.25% trypsin and 1mM EDTA for 5 min at 37°C, counted, and replated in 100-mm dishes. Only first-passage hMSCs were used in this study.

Treatment of hMSCs with adipogenic supplements

For encapsulation experiments, first-passage hMSCs were cultured separately in basic or adipogenic medium for 1 week. Basic medium consisted of MesenCult basal

medium for hMSCs (StemCell Technologies), 10% hMSC adipogenic stimulatory supplements (StemCell Technologies), and 1% antibiotic–antimycotic (GIBCO; Invitrogen). Adipogenic tissue-stimulating medium consisted of MesenCult basal medium for hMSCs (StemCell Technologies), 10% hMSC adipogenic stimulatory supplements (StemCell Technologies), and 1% antibiotic–antimycotic (GIBCO; Invitrogen). Cultures were incubated in 95% air–5% CO_2 at 37°C with medium changes every 3–4 days.

To demonstrate the adipogenic process in monolayer culture, first-passage hMSCs were cultured separately in basic or adipogenic medium for 4 weeks and stained with oil red O (Sigma). Cultures were compared for positive oil red O staining indicating fat deposition in the culture.

Hydrogel/photoinitiator solution preparation and construct fabrication

To prepare the hydrogel solution, PEGDA (MW 3400; Shearwater Polymers/Nektar AL, Huntsville, AL) was dissolved in sterile PBS supplemented with penicillin (100 U/mL) and streptomycin (100 μg/mL) (GIBCO) to a final solution of 10% (w/v). A photoinitiator, 2-hydroxy-1-[4-(hydroxyethoxy)phenyl]-2-methyl-1-propanone (Ciba Specialty Chemicals, Tarrytown, NY), was added to the PEGDA solution to obtain a final photoinitiator concentration of 0.05% (w/v).

After 1 week of incubation in either basic or adipogenic medium, first-passage hMSCs were trypsinized with 0.25% trypsin and 1 mM EDTA for 5 min at 37°C, counted, and resuspended separately in PEGDA polymer–photoinitiator solution at a density of 5×10^6 cells/mL. To prepare each construct, a 150-μL aliquot of cell–polymer–photoinitiator suspension was loaded into 9-mm diameter plastic inserts (caps of 1.5-mL microcentrifuge tubes) (Fisher Scientific, Hampton, NH). After loading, molds carrying cell–polymer–photoinitiator suspensions were exposed to long-wave, 365-nm ultraviolet light (Glow-Mark, Blauvelt, NY) at an intensity of ~4 mW/cm^2 for 5 min. The photopolymerized constructs were then removed from the mold, washed twice with sterile PBS, and transferred to 6-well culture plates (Fisher Scientific) with the corresponding, basic or adipogenic, medium.

In vitro *incubation and* in vivo *implantation of constructs*

For the *in vitro* experiment, hydrogel constructs encapsulating hMSC-derived adipogenic cells ($n = 12$) were incubated with adipogenic medium (described above), whereas hydrogel constructs encapsulating untreated hMSCs ($n = 12$) were incubated with basic medium (described above). All cultures were incubated in 95% air–5% CO_2 at 37°C for 4 weeks, changing to fresh medium every 3–4 days.

For the *in vivo* experiment, polymerized hydrogel constructs encapsulating adipogenic medium-treated hMSCs ($n = 8$), constructs encapsulating hMSCs not exposed to adipogenic tissue-inducing medium ($n = 4$), and control constructs with no cells ($n = 4$) were removed from the molds and washed twice with sterile PBS. All constructs were implanted in subcutaneous pockets in the dorsum of severe combined immunodeficient (SCID) mice (four constructs per mouse, two experimental and two control) (Harlan, Indianapolis, IN), prepared by blunt dissection, under general anesthesia with intraperitoneal injection of ketamine (100 mg/kg) plus xylazine (5 mg/kg). Animal experiments had received the appropriate approval from the institutional animal care committee.

Cell viability assay

Cell viability was assessed in 4-week *in vitro*-incubated constructs, using a LIVE/DEAD viability/cytotoxicity kit (L-3224; Molecular Probes, Eugene, OR) according to the manufacturer's recommended protocol. Calcein (a fluorescent dye) diffuses through cell membrane and reacts with intracellular esterase to produce green fluorescence, whereas ethidium homodimer (a fluorescent dye) diffuses only through damaged cell membrane and bonds to nucleic acids to produce red fluorescence. After cell labeling, samples were viewed under an inverted optical microscope (Leica Microsystems, Wetzlar, Germany) equipped with a longpass dual-emission fluorescent filter (Chroma Technology, Rockingham, VT).

Histological analysis

After a 4-week *in vitro* incubation and *in vivo* cultivation in the dorsum of SCID mice, constructs were embedded in optimal cutting temperature (O.C.T.) tissue-freezing mixture (IMEB, Chicago, IL) and 10-μm sections were cut by standard histological technique. Sections were stained with oil red O (Sigma), mounted with glycerol gelatin (Sigma), and viewed under a light microscope (Leica Microsystems).

RNA extraction and reverse transcription-polymerase chain reaction

Total RNA was extracted from harvested *in vivo* hydrogel constructs ($n = 3$), using RNeasy purification reagent (Qiagen, Valencia, CA). The constructs were homogenized (pellet pestle mixer; Kimble/Kontes, Vineland, NJ) in 1.5-mL microcentrifuge tubes containing 1.0 mL of TRIzol reagent (Invitrogen). Reverse transcription (RT) and polymerase chain reaction (PCR) were performed with an RT-PCR core kit (Applied Biosystems GeneAmp PCR system; GenTech Scientific, Arcade, NY). Two-microliter aliquots of the resulting cDNA were amplified in 50-μL volumes. Specific human primers of

the adipocyte-specific peroxisome proliferator-activated receptor γ2 (PPAR-γ2) gene (sense, 5′-GCTGTTATG-GGTGAAACTCTG-3′; antisense, 5′-ATAAGGTG-GAGATGCAGGCTC-3′) and the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (sense, 5′-CCCATGTTCGTCATGGGTGT-3′; antisense, 5′-TGGTCATGAGTCCTTCCACGATA-3′) were used. PCR was performed for 30 cycles, with each cycle consisting of denaturing at 95°C for 1 min, annealing at 56°C for 2 min, and elongating at 72°C for 3 min, with an additional 7-min incubation at 72°C after completion of the last cycle. To exclude possible contamination of genomic DNA, PCR was also applied to reactions without RT. The amplified complementary DNA was electrophoresed through a 1.5% agarose gel in the presence of ethidium bromide, and photographed under ultraviolet light.

RESULTS

All surgical procedures were uneventful. The SCID mice tolerated the *in vivo* implantation procedures well, without major complications. All results described below were consistent among all samples in the same category.

Gross examination of tissue-engineered adipose tissue constructs

After 4 weeks of *in vivo* implantation and *in vitro* incubation, both experimental constructs (encapsulating hMSC-derived adipogenic cells) and control constructs (encapsulating untreated hMSCs or no cells) maintained the physical shape and dimensions of the original plastic mold (Fig. 1A). On physical manipulation, all constructs were resilient, and retained the original consistency of the hydrogel material. The *in vivo* tissue-engineered adipogenic constructs (Fig. 1B) appeared light yellow in color and photo-opaque in contrast to transparent control constructs containing hMSCs treated with basic medium (no adipogenic tissue-inducing supplement) (Fig. 1C) and harvested control constructs with no cells encapsulated (Fig. 1D). There was no statistically significant difference in the average diameters of harvested experimental and control constructs ($p > 0.05$) (Fig. 1E).

Cell viability and in vitro *adipogenic potential of hMSCs in monolayer cultures and hydrogel constructs*

Human MSC monolayer cultures incubated with adipogenic medium for 4 weeks demonstrated positive reaction to oil red O staining, indicated by the red color (Fig. 2A), in comparison with negative oil red O staining of hMSCs cultured in monolayer with basic medium (no adipogenic supplement) (Fig. 2B). Oil red O is a lysochrome (fat-soluble dye) with deeper red color, pre-

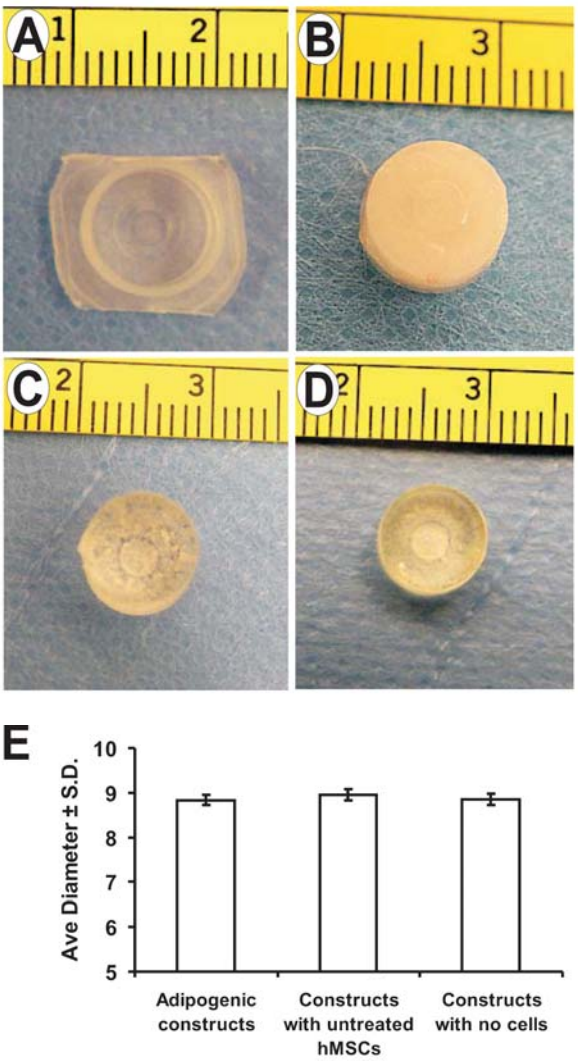

FIG. 1. Gross appearance of adipogenic constructs, tissue engineered from adult human stem cell-derived adipogenic cells, and control constructs after 4 weeks of subcutaneous implantation in the dorsum of immunodeficient mice. (**A**) Plastic mold used during fabrication of experimental and control hydrogel constructs. (**B**) Representative poly(ethylene glycol) diacrylate (PEGDA) tissue-engineered adipogenic construct encapsulating adipogenic medium-treated human mesenchymal stem cells (hMSCs) retained shape and dimensions of the original mold. (**C**) Representative *in vivo*-harvested control construct encapsulating untreated hMSCs. (**D**) Harvested control construct without any cells encapsulated. Note the dimensional resemblance of all constructs (with or without cells) to the original mold. The tissue-engineered adipogenic construct is light yellow and photo-opaque (**B**), in contrast to pale and transparent control constructs (**C** and **D**). (**E**) Average diameters (±SD) of *in vivo*-harvested adipogenic constructs ($n = 8$), control constructs with encapsulated hMSCs unexposed to adipogenic tissue-inducing supplement ($n = 4$), and negative control constructs encapsulating no cells ($n = 4$). There were no statistically significant differences in average diameters among the three types of harvested constructs ($p > 0.05$).

dominantly used for demonstrating triglycerides in frozen sections.[62] Similarly, hydrogel constructs encapsulating adipogenic medium-treated hMSCs showed positive staining with oil red O after 4 weeks of *in vitro* incubation in PEG-based hydrogel in comparison with a lack of oil red O staining in PEG-based hydrogel encapsulating nonadipogenic treated hMSCs (Fig. 2C and D). After 4 weeks incubation, the majority of the encapsulated cells were viable, as indicated by the green fluorescent labeling with calcein (Fig. 2E).

Tissue-engineered adipogenic constructs in vivo

Histological and RT-PCR analyses demonstrated *de novo* formation of adipose tissue in the hydrogel constructs encapsulating adipogenic medium-treated hMSCs after 4 weeks of *in vivo* cultivation in the dorsum of SCID mice (Fig. 3). Adipogenic constructs showed positive oil red O staining (Fig. 3A) relative to the control constructs encapsulating untreated hMSCs (Fig. 3B) or those with no cells encapsulated (Fig. 3C). In addition, control constructs with no cells encapsulated showed no signs of host cellular invasion but with the appearance of intact hydrogel borders surrounded by host fibrous capsule (Fig. 3C). Cell-seeded constructs also showed fibrous capsule formation (data not shown). The adipocyte-specific PPAR-γ2 gene was positively expressed in the adipose tissue constructs encapsulating adipogenic medium-treated hMSCs (Fig. 3D). The RT-PCR experiments were repeated and showed consistent results among the examined experimental and control constructs ($n = 3$).

DISCUSSION

The present findings demonstrated that the predefined shape and dimensions of adipogenic hydrogel constructs encapsulating human mesenchymal stem cell (hMSC)-derived adipogenic cells were well retained after 4 weeks of *in vivo* implantation. Volume retention of tissue-engineered adipogenic constructs is a necessary demonstration of the potential therapeutic value of this approach, in consideration of shape and dimension requirements associated with soft tissue augmentation and reconstruction. There appears to be considerable room for improvement over the reported 40–60% volume loss associated with conventional grafting procedures with autologous fat tissue transplantation.[2] Volume retention by the present hydrogel system encapsulating adipogenic cells derived from hMSCs, albeit in the presently tested 1-month duration, demonstrates the promise of the present hydrogel–cell encapsulation system for potential clinical application in soft tissue reconstruction and augmentation procedures. An added advantage of the present PEGDA hydrogel system is its ease for shape and dimension manipulation via *in situ* transdermal photopolymerization on subcutaneous implantation or injection.[44,56,57] The present PEGDA system has been demonstrated to retain the shape and dimensions of a human mandibular condyle encapsulating MSC-derived chondrogenic and osteogenic cells for up to 8 weeks of *in vivo* implantation.[56,57]

An increasing number of hydrogel polymers have been utilized for multiple tissue-engineering approaches.[2,7,10,34–51,61,63–67] Previous work has demonstrated that PEGDA supports the viability and cell differentiation of MSCs into multiple mesenchymal lineages.[57–60] Because of its high water content, hydrogel materials possess superior diffusion properties and provide the encapsulated cells with an extracellular matrix-like environment.[36,53,61] The somewhat slow degradation rate of PEG-based hydrogel[68] appears to be advantageous in its application in adipose tissue engineering in that the original shape and dimension of the tissue-engineered adipose tissue need to be maintained. However, a tailored hydrogel degradation rate is necessary to coincide with continuing formation of adipose tissue by delivered MSC-derived adipocytes or resident (host) adipocytes.

The presently demonstrated adipogenic potential of MSCs is consistent with previous studies designed to explore their multipotentiality.[31,69,70] The present parallel approach to determine the adipogenic potential of hMSCs both *in vitro* and *in vivo* is advantageous in elucidating that a relatively short period, only 1 week, of *in vitro* exposure of hMSCs to adipogenic tissue-inducing supplement was sufficient for hMSCs to retain their adipogenic potential for up to 4 weeks of *in vivo* implantation. Adult mesenchymal stem cells, which can be obtained via currently practiced clinical approaches with relative ease, have proven to be a well-established source for multiple mesenchymal tissue-forming cell lineages, including adipocytes.[30–32] In addition to bone marrow stroma, an increasing number of connective tissues have been reported to contain such multipotent cells.[32,33,70–77] Because of their high expandability in culture and established protocols for multlineage differentiation, adult MSCs have been increasingly utilized as the cell source for multiple cell-based tissue-engineering approaches.[3,10,13,56,57,60,78–82] Thus, the use of adult hMSCs to engineer adipose tissue implants in the present approach is aligned with the ultimate therapeutic tissue-engineering approaches.[13,69–71]

The present *in vivo* adipogenesis data are consistent with and indirectly supported by several previous approaches.[2,7,10,50] For example, peptide-linked alginate implants supported the adhesion and proliferation of seeded sheep preadipocytes, and adipose tissue formation.[50] Seeding dermal fibroblasts in alginate implants leads to significant ingrowth of fibrovascular stroma with cells thoroughly distributed within the gel.[7] Maintenance

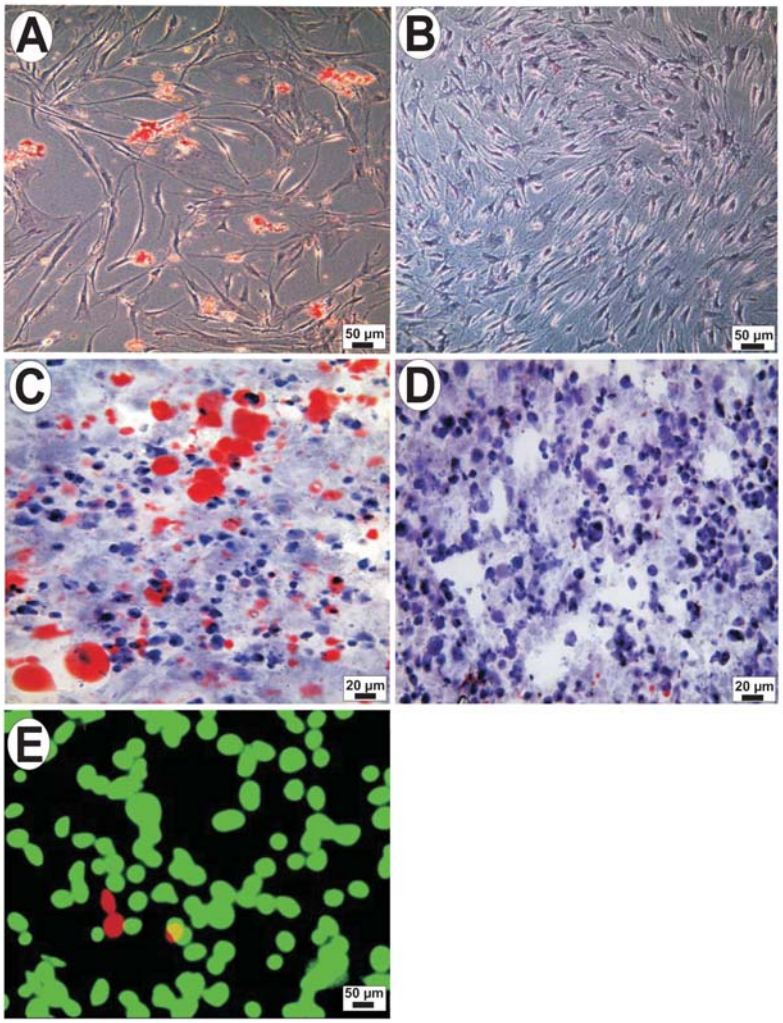

FIG. 2. Adipogenic potential of *in vitro*-incubated human mesenchymal stem cells (hMSCs) in monolayer cultures and in hydrogel constructs. (**A**) Representative positive staining with oil red O of monolayer-cultured hMSCs after 4 weeks of incubation in adipogenic tissue-inducing medium. (**B**) Representative control monolayer culture of hMSCs without adipogenic tissue-inducing treatment, demonstrating negative reaction to oil red O staining after incubation in basic culture medium for 4 weeks. (**C**) Representative fat deposits demonstrated by positive staining with o red O for hydrogel constructs encapsulating adipogenic medium-treated hMSCs and incubated *in vitro* for 4 weeks in adipogenic medium. (**D**) Representative control construct encapsulating hMSCs without adipogenic tissue-inducing treatment, showing negative reaction to oil red O staining after *in vitro* incubation for 4 weeks in basic culture medium. (**E**) Representative photomicrograph of LIVE/DEAD cell fluorescence labeling of hMSCs encapsulated in a PEGDA construct and incubated *in vitro* for 4 weeks. Live cells are green (calcein), whereas dead cells appear red (ethidium homodimer).

of volume of the alginate constructs ranged between 19 to 88% after 8 weeks of subcutaneous implantation in rats, with the highest percentage of volume maintenance on seeding fibroblasts in alginate and *in situ* solidification after subcutaneous injection.[7] In contrast, the present approach utilized preconditioned adipose tissue-forming cells from human bone marrow-derived mesenchymal stem cells in a biocompatible hydrogel with no apparent volume loss observed in the tissue-engineered constructs after 4 weeks of *in vitro* incubation and *in vivo* implantation.

The control hydrogel constructs with no cells encapsulated demonstrated an intact hydrogel border and host tissue fibrous capsule surrounding the implants after 4 weeks of *in vivo* implantation (see Fig. 3C). This finding indicates that host tissue cells have not invaded the constructs and that the presented adipose tissue formation is to be primarily attributed to encapsulated adipogenic cells derived from the mesenchymal stem cells. Previous ap-

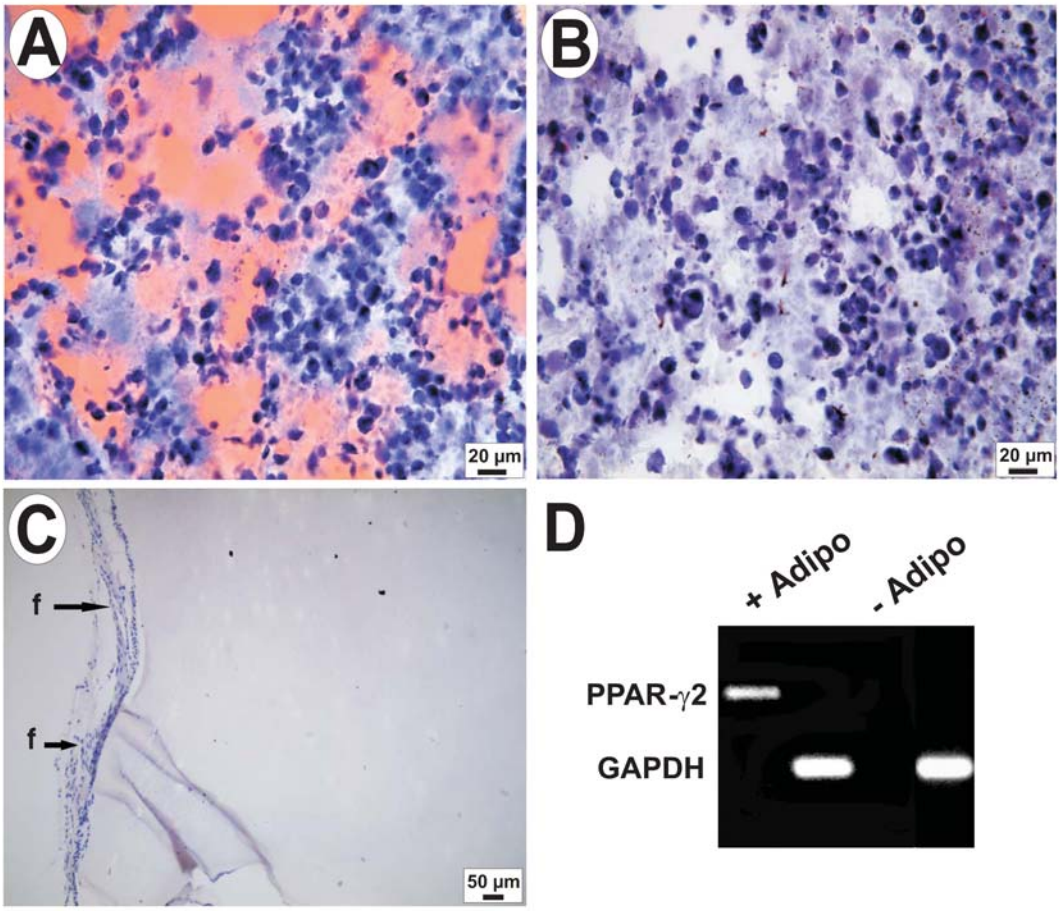

FIG. 3. *In vivo* adipogenesis induced by tissue-engineered hydrogel constructs encapsulating human mesenchymal stem cell (hMSC)-derived adipogenic cells. (**A**) Representative positive oil red O staining of tissue-engineered hydrogel construct encapsulating adipogenic-treated hMSCs and implanted in the dorsum of an immunodeficient mouse for 4 weeks. (**B**) Representative micrograph of control hydrogel constructs encapsulating hMSCs without adipogenic-inducing treatment and implanted in the dorsum of immunodeficient mice for 4 weeks demonstrates no fat deposition as evident by negative reaction to oil red O staining. (**C**) Representative micrograph of control hydrogel constructs encapsulating no cells shows a negative reaction to oil red O staining. The implanted control construct was characterized by the absence of host cell invasion and was surrounded by a host fibrous tissue capsule (f). (**D**) RT-PCR analysis showing expression of adipocyte-specific gene (PPAR-γ2) in constructs harvested after 4 weeks of subcutaneous implantation in immunodeficient mice, encapsulating adipogenic-treated hMSCs (+Adipo) or hMSCs without adipogenic-inducing treatment (−Adipo) relative to a housekeeping gene (GAPDH).

proaches utilizing porous scaffolding materials with seeded adipogenic cells have not conclusively stated that the newly formed adipose tissue was from the transplanted cells or the invading host tissue cells.[48] However, it is to be determined whether host adipose invasion is facilitative or counterproductive in eventual clinical applications using stem cell-derived tissue-engineering approaches.

The present approaches need to be optimized along several fronts before application of the tissue-engineered adipose tissue constructs for reconstructive and augmentation work in patients. Only single-cell encapsulation density was studied in the current approach. Presumably, a valid question arises concerning the optimal encapsulation density of adipogenic medium-treated hMSCs to maximize the regenerative outcome of the tissue-engineered fat tissue constructs. The quality of a number of tissue-engineering approaches has been shown to be influenced by the initial seeding density of the tissue-forming cells.[83–87] The degradation rate of PEG-based hydrogel might need to be optimized to coincide with the rate of *in vivo* adipogenesis and to allow for constructive integration between the newly formed tissue and the host tissue. Several attempts were directed toward chemical modification of the degradation rate of the PEG-based hydrogel system by the addition of degradable linkages, such as polyester and phosphate groups,[88–91] to the macromere backbone. Also, growth factor delivery through controlled release technology may prove to be necessary to maintain

the phenotypic differentiation of the adipogenic cells and/or to enhance the vascularization of the newly formed tissue.[34] Finally, the positive influence of the mechanical modulation of tissue-engineered constructs observed in the case of bone and cartilage tissue engineering,[92–96] for example, may prove to be a positive influential factor in the adipogenic process as well.[97] Nonetheless, the present approach represents another step toward an alternative tissue-engineering approach for soft tissue augmentation and reconstruction by presenting a clinically valid model for adipose tissue engineering from adult human mesenchymal stem cells. Synergistic efforts in areas such as cell culture, biomechanics, cell differentiation, angiogenesis, computer modeling, and polymer chemistry are likely necessary before reaching the ultimate goal of clinical applications in patients in need of soft tissue augmentation and reconstruction.

ACKNOWLEDGMENTS

We thank Dr. Joseph Daw for critical review of the manuscript. We are grateful to all referees whose thorough and valuable comments helped improve the quality of the manuscript. Aurora Lopez and Alexandra Peptan are acknowledged for technical assistance. The present work was supported by NIH grants R01DE13964 and R01DE15391 from the National Institute of Dental and Craniofacial Research, and by grant R01EB02332 from the National Institute of Biomedical Imaging and Bioengineering.

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