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METHODS ARTICLE

Sandwiched White Adipose Tissue: A Microphysiological System of Primary Human Adipose Tissue

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White adipose tissue (WAT) is a critical organ in both health and disease. However, physiologically faithful tissue culture models of primary human WAT remain limited, at best. In this study we describe a novel WAT culture system in which primary human WAT is sandwiched between tissue-engineered sheets of adipose-derived stromal cells. This construct, called ''sandwiched white adipose tissue'' (SWAT), can be defined as a microphysiological system (MPS) since it is a tissue-engineered, multicellular, three-dimensional organ construct produced using human cells. We validated SWAT against the National Institutes of Health MPS standards and found that SWAT is viable in culture for 8 weeks, retains physiologic responses to exogenous signaling, secretes adipokines, and engrafts into animal models. These attributes position SWAT as a powerful tool for the study of WAT physiology, pathophysiology, personalized medicine, and pharmaceutical development.

Keywords: SWAT, white adipose tissue, microphysiological system, tissue engineering, fat culture

Introduction

WHITE ADIPOSE TISSUE (WAT) is a critical organ in both health and disease. As an energy reservoir and endocrine organ, WAT regulates energy homeostasis, immunity, vascular tone, and coagulation.[1] As the organ of disease in obesity, WAT overgrowth is a powerful risk factor for every leading cause of death, including heart disease, diabetes, stroke, and cancer.[2,3] Appropriately, WAT is the subject of intense study.

However, primary WAT is difficult to maintain *in vitro* because adipocytes are buoyant, terminally differentiated, and prone to rupture. Approaches for overcoming these barriers have been reported since the 1970s, utilizing suspension culture, glass coverslips, hydrogels, etc.[4–8] While these techniques were partly successful in rodent WAT, they were rarely extended to human WAT. Profound differences exist between rodent and human WAT, including anatomical distribution, metabolic activity, and response to exogenous signals. Such differences render applicability of murine studies to human health dubious, and this enduring hurdle has slowed progress in treating human disease.[9–12]

Most WAT research entirely avoids the challenges of culturing primary WAT by relying on differentiated adipocytes (diffAds). diffAds have the benefit of being produced from nonbuoyant stromal cells that are easily passaged using standard tissue culture (TC) methods. Although diffAds are usually referred to as ''adipocytes,'' they are not equivalent to mature adipocytes. Instead, as they are produced by inducing adipocyte precursors toward an adipocyte-like phenotype, diffAds more precisely model the mechanisms of adipogenesis.[13–17]

Without a well-validated *in vitro* model of mature human WAT, our ability to study physiology and disease is hampered. This challenge is not unique to WAT, and the need for physiologically faithful models of all human organ systems led to the creation of the National Institutes of Health (NIH) microphysiological system (MPS) Program.[18] Defined as tissue-engineered, multicellular three-dimensional (3D) organ constructs produced using human cells, MPSs

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are expected to accelerate bench-to-bedside translation.[18–22] As a broad multiagency effort, the NIH MPS Program also defined a rigorous set of benchmarks against which MPSs can be evaluated.

We therefore sought to leapfrog the challenges of traditional WAT culture by developing a novel, inexpensive, and feasible primary WAT MPS. We achieved this by combining clinical insights from fat grafting procedures with proven cell sheet technology. In our approach, minced primary human WAT is sandwiched between sheets of human adipose-derived stromal cells (ASCs), yielding ''sandwiched white adipose tissue'' (SWAT). SWAT fulfills the NIH MPS standards: it uses primary multicellular human sources, is viable in culture for 8 weeks, and recapitulates the native 3D architecture and cellular composition of WAT. We further demonstrate that SWAT responds to exogenous signaling, secretes adipokines, and engrafts into animal models.

Because SWAT only uses commercially available materials and does not require microchip or microfluidics expertise, it is widely available to the research community. We expect that SWAT will provide a broadly useful platform for the study of WAT physiology, pathophysiology, personalized medicine, and drug screening.

Methods

All human tissues were collected in adherence to protocols #8759 and # 9189, as approved by the IRB Office of Louisiana State University Health Sciences Center (LSUHSC).

ASC isolation

ASC lines were isolated using an established methodology.[23] Briefly, human adipose tissue was obtained from subjects undergoing elective surgery. Samples were washed in phosphate-buffered saline (PBS) then minced (using sterile razor blades) into 0.5–1 mm segments. Minced tissue was incubated in Krebs Ringer Buffer (KRB) with 1 mg/mL collagenase (Sigma) and agitated at 37°C for 30 min.

The sample was centrifuged at 1500 rpm for 5 min to separate the stromal vascular fraction (SVF) from the buoyant adipocyte layer. The pelleted SVF was washed in KRB and resuspended in Dulbecco's modified Eagle's medium (DMEM). The SVF was plated and maintained in low glucose DMEM (Life Technologies) and then it was supplemented with 10% fetal bovine serum (FBS; Life Technologies) and 1% penicillin/streptomycin solution (Life Technologies). Media was changed every other day. Cells were maintained at 37°C with 5% CO_2.

Production of SWAT cultures

Six centimeter single dishes (or six-well multidishes) were seeded at ~80% confluency with the ASCs. Base layer cell sheets were cultured on standard TC dishes (Corning), and upper layer sheets were seeded on thermoresponsive UpCell™ dishes (Nunc). Cells were grown until they were confluent. A solution of 7.5% gelatin was prepared with gelatin powder (Sigma) and 0.01 M NaOH in Hanks' Balanced Salt Solution (HBSS; Life Technologies) at 75°C. The gelatin was solidified on a plunger apparatus at room temperature at approximate volumes of 3 mL for a six-well multidish and 6 mL for a 6 cm dish. The plunger was applied to upper layer cell sheets at room temperature for 1.5 h and then it was placed in an ice water bath for an additional 1.5 h to release the sheet from the UpCell dish.

Fresh primary adipose tissue was washed with PBS and minced into 0.5–1 mm segments. Minced tissue was mixed with DMEM at an approximate ratio of 3:1 in individual aliquots and applied to the base cell sheets. Approximate total volumes of 300 and 800 μL of media/tissue mixture were used to seed six-well multidishes and 6 cm plates, respectively. Upper layer cell sheets and plungers were applied, warmed culture media was added to each plate, and the plates were incubated on a 37°C heat block for 30–35 min to release the upper sheets from the plungers (Fig. 1). For initial characterization of SWAT, including serial bright field imaging, all cellular staining, lipolysis, and *in vivo* engraftment, SWAT cultures were maintained in low glucose DMEM supplemented with 10% FBS and 1% penicillin/streptomycin solution. To facilitate long-term adipocyte transcription, SWAT cultivated for transcriptional analysis and leptin secretion was maintained in Medium 199 with Earle's Salts (Thermo Fisher) with 7 nM insulin, 30 nM dexamethasone, and 1% penicillin/streptomycin solution. All SWAT was maintained at 37°C at 5% CO_2. Media was changed every other day.

Enhanced green fluorescent protein transduction

ASCs were grown to ~80% confluence on UpCell dishes. Cells were inoculated with enhanced green fluorescent protein (eGFP)-expressing adenovirus 5 (University of Iowa Viral Vector Core) in unsupplemented DMEM at an multiplicity of infection of 200. Cells were incubated at 37°C for 4 h. Viral media was then removed, and cells were maintained in standard media.

Dissociation of WAT from SWAT cultures

To separate SWAT from ASCs in culture, SWAT cultures were enzymatically dissociated. Briefly, wells were washed in PBS, and cells were dislodged from the culture surface with a cell scrapper. Cells were suspended in 0.5 mg/mL collagenase (Sigma) in PBS, agitated at 37°C for 30–60 min, and settled 5 min at room temperature. This process allowed the buoyant adipocytes to rise in solution, while the ASCs and debris settled into lower phases. The WAT-containing upper phase was isolated, and the excess liquid under the floating WAT was removed using a 23G needle and syringe.

Propidium iodide stain

One milligram/milliliter stock solution of propidium iodide (PI; Sigma) was prepared in diH_2O; 5 μM working solution was made in PBS. SWAT plates were washed in PBS, stained in working PI solution for 30 min at 37°C, and washed again in PBS before imaging.

Nile Red stain

One milligram/milliliter stock solution of Nile Red stain (Sigma) was prepared in dimethyl sulfoxide. Working solution was prepared diluting stock 1:1000 in PBS shortly before staining. Prestained fluorescent images of SWAT plates were obtained to confirm minimal autofluorescence (data not shown). SWAT was washed in PBS and incubated

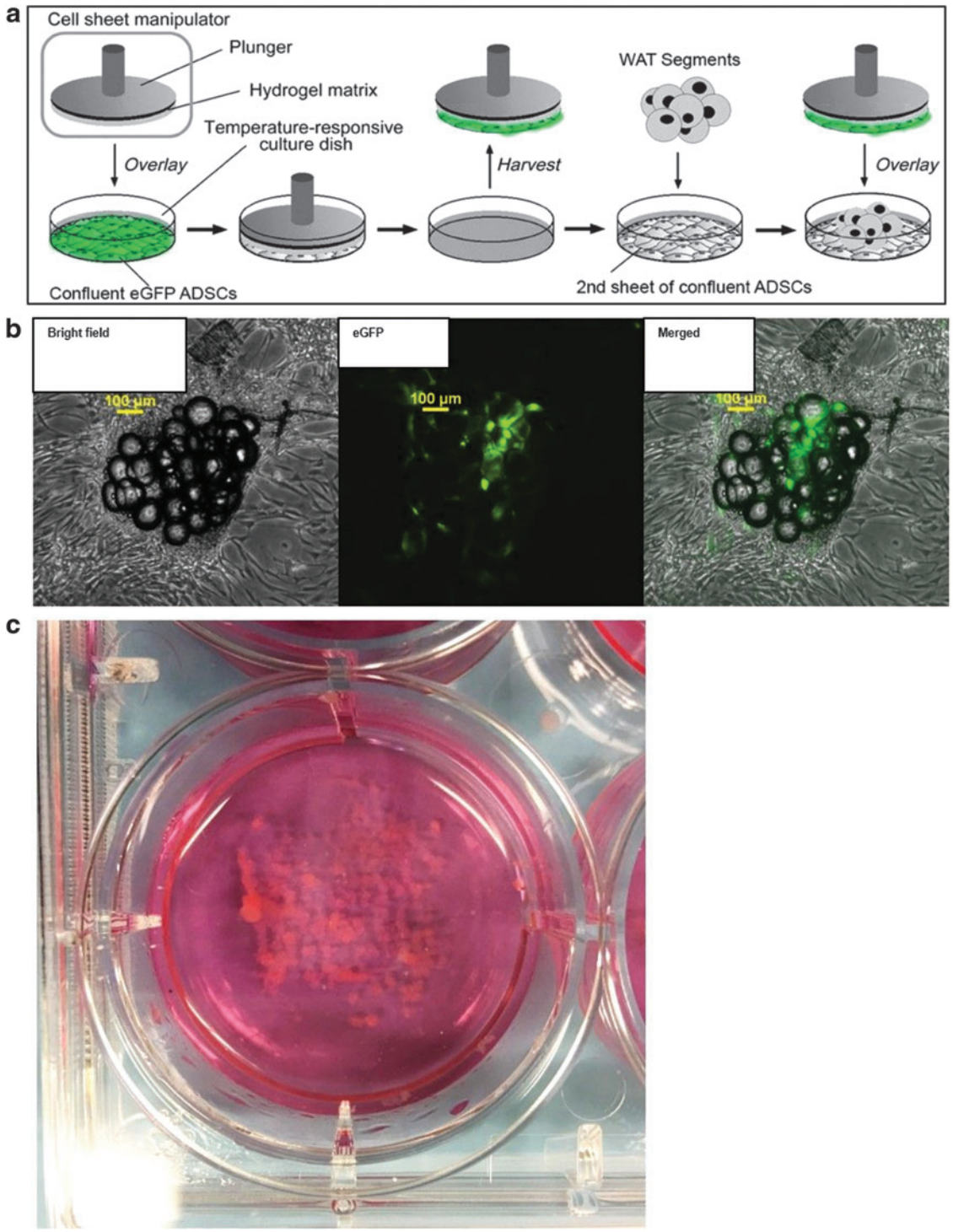

FIG. 1. The SWAT method. **(a)** Schematic of SWAT showing the transfer of a sheet of eGFP-labeled ASCs from a poly(N-isopropylacrylamide)-coated TC dish (UpCell) onto a second unlabeled sheet of ASCs grown on a standard TC dish. Sandwiched between the two ASC sheets is minced, primary human WAT. **(b)** Fluorescence microscopy demonstrating Brightfield, GFP, and merged channels of a SWAT created by the technique described in **(a)**. Scale bar = 100 µm. **(c)** Digital photograph of a representative 5-day-old SWAT cultured in a standard six-well TC plate. The large volume of WAT is stably secured to the bottom of the well. WAT, white adipose tissue; SWAT, sandwiched white adipose tissue, eGFP, enhanced green fluorescent protein; ASC, adipose-derived stromal cell; TC, tissue culture.

in working Nile Red solution for 5 min at 37°C. Nile Red solution was aspirated, SWAT was washed with PBS, and images were captured.

Oil Red O stain

A 0.35% stock solution of Oil Red O (Sigma) was prepared in Isopropanol. A working solution was prepared diluting stock solution with deionized water at a 1:1 ratio shortly before staining. SWAT plates were washed for 15 min with room temperature PBS; plates were first fixated with 3.7% paraformaldehyde for 10 min at room temperature and then fixated for an additional hour with fresh paraformaldehyde. Fixed plates were washed twice with deionized water and in 60% isopropanol for 5 min and then completely dried at room temperature. Plates were stained with working stock of Oil Red O for 20 min and then thoroughly washed with deionized water before imaging.

Immunocytochemistry

SWAT cultures were washed in PBS then fixed for 30 min at room temperature in 2% EM grade paraformaldehyde (Electron Microscopy Sciences) that was diluted in PBS. Plates were blocked with blocking buffer (5% bovine serum albumin [BSA], 0.1% saponin in PBS), for 30 min at room temperature. Plates were stained with primary antibodies against perilipin (Abcam; ab3526, 1:200 dilution), PPARγ2 (Santa Cruz; sc-166731, 1:100 dilution), and FABP4 (Abcam; ab92501, 1:1000 dilution) and stayed in condition overnight at 4°C. Secondary antibody incubations were performed for 1 h at room temperature (Abcam; Alexa Fluor 488 and 594, 1:500 dilution) with 4′,6-diamidino-2-phenylindole (DAPI; Sigma). Images were taken of either cells in diH_2O or after plates were briefly dried and coverslipped with ProLong mounting medium (Life Technologies).

RNA isolation and reverse transcription–polymerase chain reaction

RNA was extracted using RNeasy Lipid Tissue Mini Kit (QIAGEN). Dissociated WAT was suspended in 200 μL of QIAzol Lysis Reagent, and cells were homogenized with individual pestles (Kimble Kontes). An additional 800 μL of lysis reagent was added, and the homogenate was incubated for 5 min at room temperature. Two hundred microliter of chloroform was added, and the homogenate was incubated for 2 min at room temperature. Samples were centrifuged at 12,000 *g* for 15 min at 4°C, and the upper aqueous phase was taken for RNA extraction as per the manufacturer's protocol.

Sample concentration and purity were assessed using NanoDrop (Thermo Fisher), and samples were stored at −80°C. All RT-qPCR was performed on an iCycler (Bio-Rad) using a QuantiTect SYBR Green RT-PCR Kit (QIAGEN). RNA was diluted for a final input of 5 ng per reaction. Samples were spiked with 10 nM fluorescein (Sigma), and reactions were performed with listed primer sequences. The thermal cycler conditions were as follows: reverse transcription at 50°C for 30 min, polymerase activation at 95°C for 15 min, 40 three-step cycles of denaturation (95°C for 15 s), annealing (53°C for 30 s), and extension (72°C for 30 s). Data were collected using iQ5 software (Bio-Rad).

WAT protein quantification

WAT cellular protein was quantified for normalization of secreted adipokines and lipolysis. For SWAT cultures and minced fat controls, WAT was dissociated as previously described. Once WAT was isolated, 200 μL of 0.1% Triton X-100 in PBS was added to solubilize proteins, and the samples were homogenized with individual pestles. For ASC controls, 200 μL of 0.1% Triton X-100 in PBS was added to the plate directly, and cells were scrapped for homogenization. Homogenates were then centrifuged 12,000 *g* at 4°C for 5 min to separate cellular debris from solubilized protein. Samples were mixed with Bradford Reagent (Bio-Rad), and protein concentration was measured at 595 nm on a multiwall plate reader (Bio-Rad).

Secreted adipokine quantification

Levels of secreted leptin were measured using a commercial ELISA Kit (Abcam). Supernatants were collected from SWAT cultures and centrifuged 1500 rpm at 4°C for 10 min to clear cellular debris. The interphase between the cellular pellet and the lipid upper layer was taken and stored at −80°C. For ASC controls, two ASC cell sheets were stacked in the absence of WAT (data not shown). Media changes were performed in parallel with SWAT cultures. After collection of supernatant, cells were harvested for protein extraction and quantified as previously described.

Lipolysis

SWAT cultures were serum starved in 1% FBS DMEM for 1 h and then WAT was dissociated as previously described. Isolated WAT was incubated in 300 μL of 2% fatty acid-free BSA in HBSS, either with 100 μM forskolin 1 μM epinephrine (treated samples) or without additives (negative samples). WAT was incubated for 3 h at 37°C, and the media was collected for analysis. Glycerol levels were measured by adding 50 μL of collected media with 150 μL of Free Glycerol Reagent (Sigma) in a 96 well plate. Plates were then incubated for 10 min at 37°C and measured at 540 nm on a multiwall plate reader (Bio-Rad). For subject-matched primary fat controls, minced WAT was taken after seeding the SWAT plates and incubated directly in 300 μL of media (either negative or treated). The normal assay protocol was then completed.

Implantation of cultured WAT in a mouse model

All animal work was performed in adherence with protocol #3285 as approved by the Institutional Animal Care and Use Committee Office at LSUHSC. Mice utilized were 4- to 6-week-old NSG-eGFP mice (Jackson; Strain: 021937). SWAT cultures were maintained for 10 days, and WAT was dissociated as previously described. Under sterile conditions, an incision was made in the mouse dorsal subcutaneous tissue. Isolated WAT was drawn into a 1 mL syringe with an 18G needle; the needle was then removed, and the WAT was applied into the subcutaneous pocket. Mice were sutured and monitored 10 days postsurgery. On day 10, animals were sacrificed, and implanted tissue was recovered.

Immunohistochemistry

Tissue samples were fixed in zinc-buffered formalin (Richard-Allan) for 1 h and transferred to PBS. Slides with paraffin-embedded tissue sections were baked at 60°C for 45 min, deparaffinized, and rehydrated. A pressure cooker (Cuisinart) was utilized to perform heat-induced epitope retrieval in Sodium Citrate Buffer (10 mM Sodium Citrate, 0.05% Tween 20, pH 6). Slides were agitated in a blocking buffer, for 30 min at room temperature. A secondary blocking step was performed, for 1 h at room temperature, with goat anti-mouse F(ab) (Abcam; 1:100 dilution) to block any endogenous mouse immunoglobulin G present in tissue.

Primary antibody incubations were performed overnight at 4°C with antibodies against GFP (Chik anti-GFP; Fisher; A10262, 1:100 dilution) and perilipin (Ms anti-perilipin; Santa Cruz; sc-390169, 1:100 dilution). The chosen mouse anti-perilipin antibody was experimentally determined to have no reactivity in mouse tissue (data not shown). Secondary antibody incubations were performed for 1 h at room temperature (Abcam; Alexa Fluor Gt anti-Chik 488 and Gt anti-Ms 594, 1:500 dilution) with DAPI (Sigma). Sections were coverslipped with ProLong mounting medium and imaged.

Statistical analyses

GraphPad Prism was used to conduct all statistical analyses.

Statistical significance between two groups was determined using a two-tailed student's *t*-test; *p*-values of $<$ or $= 0.05$ were considered significant. All values in the text and figures are presented as mean ± standard error.

Results

Subject demographics

SWAT was created using WAT from 62 of 62 enrolled human subjects (100%) (Fig. 1). Donor sites were subcutaneous WAT from multiple anatomical areas: abdomen, chest, gluteus, and limbs. Subjects' ages ranged from 26 to 61 years (mean 42.3 years); body mass index ranged from 23.1 to 39.3 (mean 28.9); 84% were female; and 13% were diabetic.

Previous WAT culture methods led to rapid adipocyte death; SWAT remains viable for 7.6 weeks in culture

Serial brightfield imaging of individual WAT clusters ($n = 12$) for 7 weeks demonstrated mild morphological changes with no adipocyte dedifferentiation, fragmentation, or adipocyte death (Fig. 2). This long term was further demonstrated using three different positive and negative methods: PI staining to demonstrate no cell death and Nile Red and Oil-Red-O staining to demonstrate lipid retention and intact adipocyte cell membranes, both strongly indicative of live adipocyte morphology. PI staining of 53-day-old SWAT demonstrated no PI^+ adipocytes (Fig. 3A). Nile Red staining revealed large unilocular adipocytes, with no leakage of neutral lipids, suggesting viable adipocytes with intact cell membranes. This stain also demonstrated no diffAds in the surrounding ASC sheets (Fig. 3B). Oil Red O

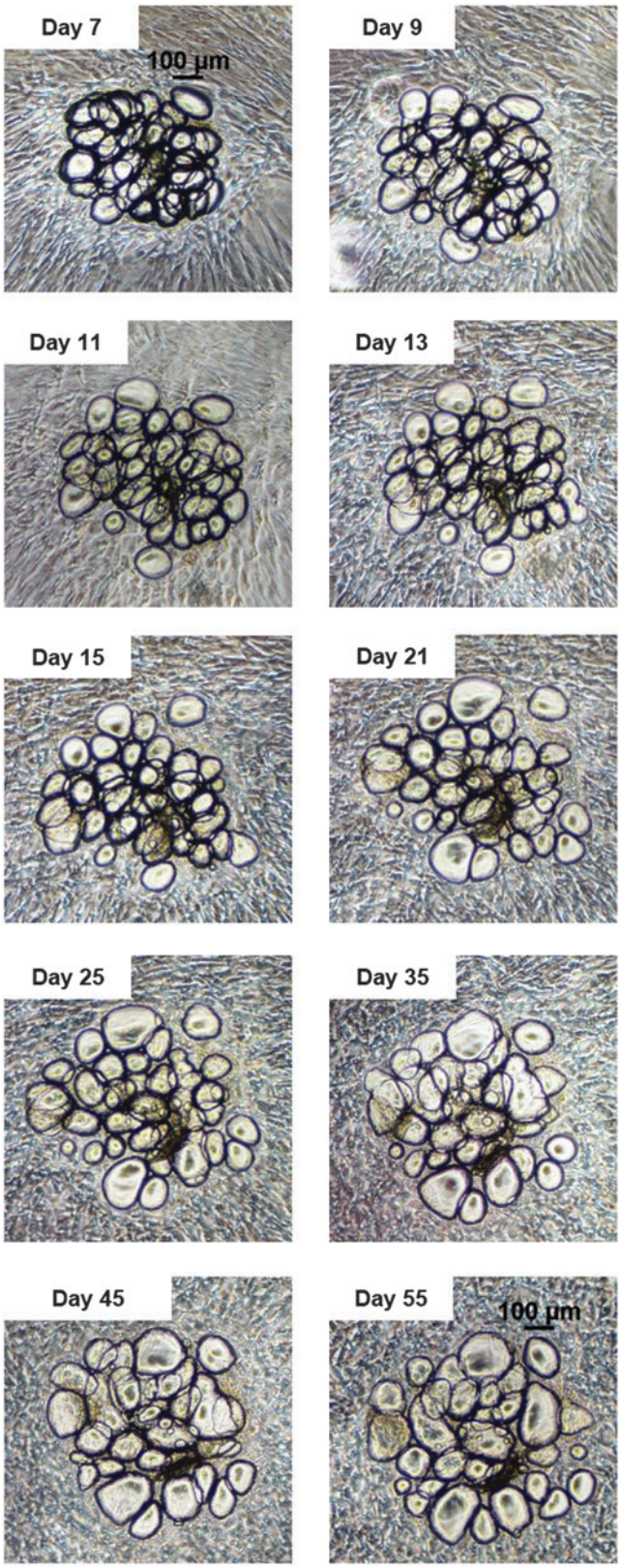

FIG. 2. SWAT remains viable at 8 weeks in culture. Serial Brightfield imaging of a single SWAT cluster over 55 days demonstrates no morphologic changes consistent with cell death. Scale bars = 100 µm.

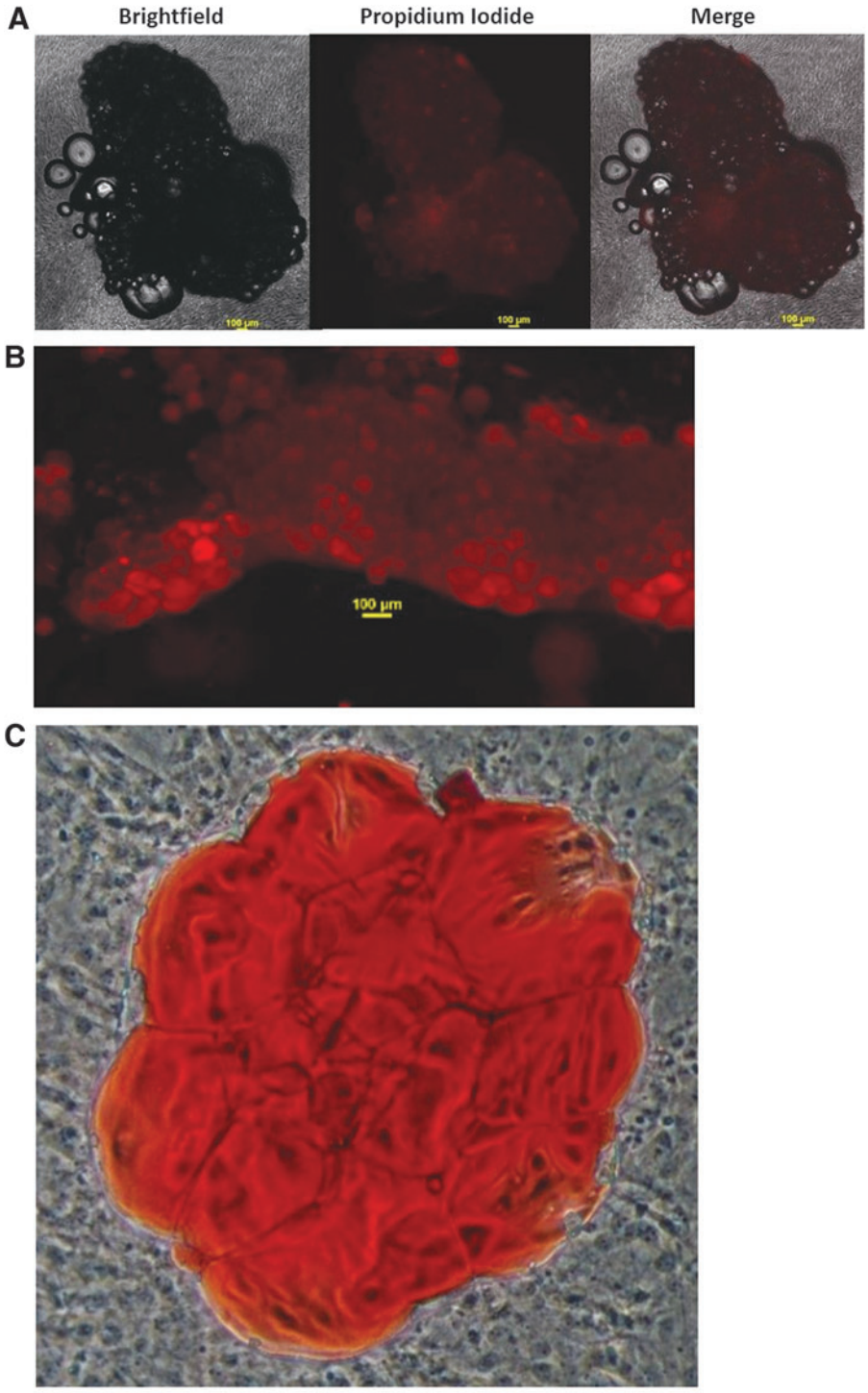

FIG. 3. SWAT remains viable at 55 days. **(A)** PI-staining of SWAT cultured for 53 days reveals complete PI exclusion, indicating no adipocyte death. Scale bar = 100 µm. To confirm that the PI+ cells were adipocytes, the cultured WAT was collagenase dissociated to isolate adipocytes and PI stained. **(B)** Nile Red staining demonstrates staining restriction to SWAT cultured for 15 days, indicating viable adipocytes. Surrounding stromal cells did not stain. 40× magnification. Scale bar = 100 µm. **(C)** Oil Red O staining of a 51-day-old SWAT demonstrates restriction of neutral lipids to the adipocytes, indicating long-term stability of adipocyte cell membranes. PI, propidium iodide.

staining demonstrated fully intact adipocyte cell membranes (Fig. 3C).

SWAT preserves mature adipocyte physiology as determined by transcriptional profiles, protein expression, and both basal and stimulated endocrine function

To evaluate the transcriptional profile of SWAT, we extracted total RNA from adipocytes isolated after 24 h, 14 days, and 28 days in SWAT culture ($n = 4$ donors). Using RT-qPCR, we determined expression levels relative to subject-matched primary WAT for six key adipocyte genes: *PPARG*, *FABP4*, *LPL*, *CEBPA*, *HSL*, and *ADIPOQ* (Fig. 4; Table 1 and Supplementary Table S1; Supplementary Data are available online at www.liebertpub.com/tec).[13] All genes were expressed at robust levels but trended downward over 4 weeks, suggesting that culture conditions could be further optimized. Expression levels were normalized to reference gene *ACTB* as it showed the least variability compared to

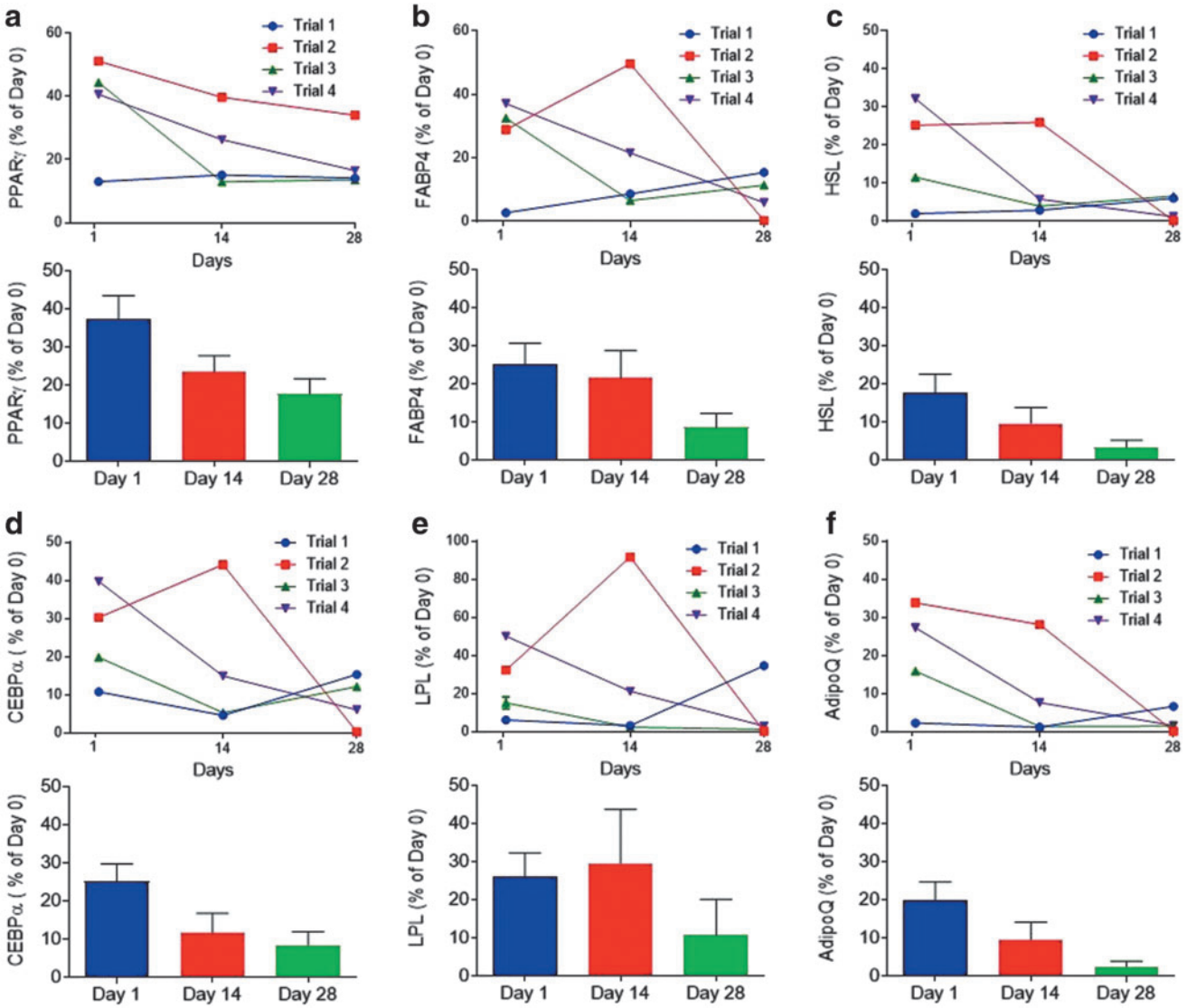

FIG. 4. SWAT maintains key adipocyte identity gene expression. SWAT clusters were collagenase digested after 1-, 14-, and 28 days in culture and gene expression determined by RT-qPCR. RT-qPCR demonstrates that 1-day-old and 14-day-old SWAT preserve high levels of **(a)** *PPARG*, **(b)** *FABP*, **(c)** *HSL*, **(d)** *CEBPA*, **(e)** *LPL*, and **(f)** *ADIPOQ*. *ACTB* was used as reference gene (See Supplementary Data). RT-qPCR, quantitative reverse transcription–polymerase chain reaction.

other adipocyte-specific reference genes (*LRP10*, *RPLP0*; data not shown).[24]

As a measure of basal endocrine function, we measured basally secreted leptin levels using enzyme-linked immunosorbent assay. SWAT preserved this function at 24 h, 2 weeks, and 4 weeks in culture ($n=4$ donors, Fig. 5a). ASC sheets without WAT secreted neither hormone (data not shown).

To demonstrate intact secretory function more rigorously, we stimulated SWAT after 0, 1, and 5 days in culture with epinephrine and forskolin, then quantified glycerol release as an end product of lipolysis (Fig. 5b, $n=4$). With stimulation, glycerol release significantly increased in all three groups: primary WAT released 82 ± 46% more ($p=0.01$), 1-day SWAT released 577 ± 387% more ($p=0.02$), and 5-day SWAT released 348 ± 343% more ($p=0.03$).

While both 1- and 5-day SWAT demonstrated a greater response to adrenergic stimulation than primary WAT, only 1-day SWAT was significantly higher ($p=0.04$). The greater responsiveness observed in SWAT may be a result of higher basal glycerol release in primary WAT: mean baseline glycerol levels for primary WAT, 1-day SWAT, and 5-day SWAT prestimulation were 3.0 ± 0.9, 0.7 ± 0.2,

Table 1. Mean Expression Levels ± Standard Error of Mean in Table Format

Gene	*Day 1 (% of day 0)*	*Day 14 (% of day 0)*	*Day 28 (% of day 0)*
PPARG	37.33 ± 6.20	23.52 ± 4.25	17.69 ± 3.70
FABP4	25.42 ± 5.80	21.62 ± 7.11	8.60 ± 3.67
HSL	17.77 ± 4.77	9.70 ± 4.11	3.40 ± 1.86
CEBPA	25.15 ± 4.57	13.40 ± 5.51	9.64 ± 3.78
LPL	25.92 ± 6.43	29.60 ± 14.12	10.72 ± 9.41
ADIPOQ	19.80 ± 4.85	9.57 ± 4.58	2.2.46 ± 1.43

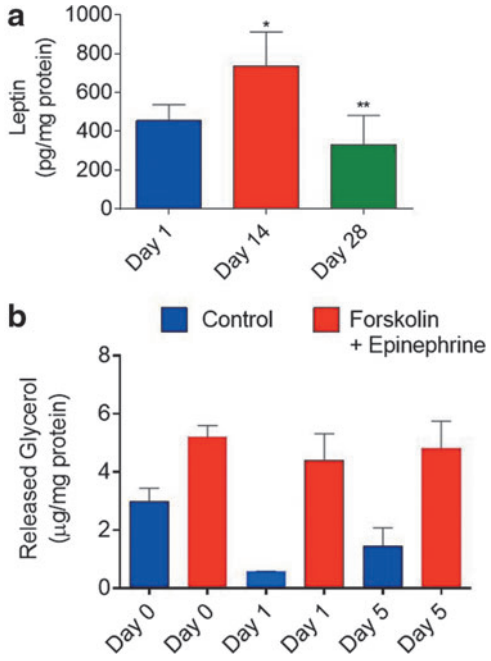

FIG. 5. SWAT basally secretes adipokines and responds physiologically to insulin and catecholamine stimulation. **(a)** Enzyme-linked immunosorbent assay quantification of 24-h basal leptin secretion shows no significant change after 28 days in culture. **(b)** Adrenergic stimulation with epinephrine and forskolin generates a significant glycolytic response in freshly harvested primary WAT (1.7×) and subject-matched SWAT at 1- and 5 days in culture. Day 1 SWAT responded to catecholamine stimulus with a 6.3-fold increase over basal glycolysis, while Day 5 SWAT demonstrated a 3.3-fold increase over basal glycolysis. Stimulated glycolysis was not significantly different in SWAT compared to matched fresh WAT ($p=0.53$, $n=4$). * indicates a p-value <0.09 and ** indicates a p-value <0.25.

and $1.4\pm0.9\,\mu g$ glycerol/mg total protein, respectively. Poststimulation, mean glycerol levels were very similar across all three groups: primary WAT 5.2 ± 0.8, 1-day SWAT 4.4 ± 1.9, and 5-day SWAT $4.8\pm1.8\,\mu g$ glycerol/mg total protein. In summary, SWAT demonstrated no diminishment in lipolytic capacity at either 1 or 5 days, but trended toward lower basal lipolysis compared to primary WAT.

Using immunocytochemistry, we showed that SWAT preserved protein expression of mature adipocyte markers PLIN, FABP4, and PPARγ. Confocal microscopy of 12-day-old SWAT revealed a clean PLIN signal with localization to adipocyte cell surface (Supplementary Fig. S1a). BODIPY counterstaining showed that FABP4 localized to adipocyte cytoplasm (Supplementary Fig. S1b), and DAPI counterstaining demonstrated PPARγ localization to nuclei (Supplementary Fig. S1c).

SWAT can be reimplanted in vivo *and reharvested*

As a final demonstration of preserved complex whole-tissue function, we transplanted 10-day-old SWAT into immunocompromised eGFP-expressing mice [NOD.Cg-Prkdcscid Il2rgtm1Wjl Tg(CAG-EGFP)1Osb/SzJ; Jackson Laboratories] ($n=4$). The transplanted SWAT was successfully recovered 10 days after transplantation (Fig. 6A). Adipocytes from the recovered SWAT ranged from 50 to 120 µm in diameter, which is the expected size for primary human adipocytes (Fig. 6B). Furthermore, the recovered adipocytes were universally $eGFP^-$ and intermittently human $PLIN^+$ (31.3%), whereas 100% of background stromal and support cells were $eGFP^+$ and human $PLIN^-$. By contrast, adipocytes from contralateral fat depots harvested from the same mice were much smaller (20 to 40 µm diameters) and 100% were $eGFP^+$/human $PLIN^-$ (Fig. 6C). As controls, we transplanted ASC sheets without WAT ($n=4$); these did not yield any recoverable tissue (data not shown).

Discussion

Stable, *in vitro* culture of primary human WAT is important because long-term viability is a proxy for cellular health. Populations of progressively dying cells produce unreliable data and misleading results. Long-term culture of WAT has persisted as an unsolved challenge because adipocytes are buoyant and prone to death. These qualities precluded the use of standard TC surfaces.[25–27] Attempts to embed adipocytes in extracellular matrices (ECMs) preserved rodent adipocyte viability but, to our knowledge, have not been successfully extended to human WAT.[5,7]

SWAT was designed to overcome these barriers by combining insights from clinical fat grafting and tissue engineering. The success of fat grafting in reconstructive surgery showed that, given the right environment, macroscopic WAT segments survive and engraft.[28,29]

Since ASCs are the WAT stromal population and help regulate WAT physiology,[30,31] we hypothesized that encapsulating minced WAT with ASCs would provide an appropriate environment. Doing so would also allow SWAT to meet the definition of an MPS as an ''*in vitro*, three-dimensional organ system from human cells on bioengineered platforms that mimic *in vivo* tissue architecture and physiological conditions.'' In this study, we proved that hypothesis: SWAT preserves the transcriptional profiles, endocrine function, and whole-tissue function of mature human WAT. Our work validates SWAT as the first WAT MPS to meet the standards of the NIH MPS program.[20]

In cell sheet engineering, first reported by Hirose *et al.* in 2000,[32] cell sheets are grown on and released from thermoresponsive substrates such as poly(*N*-isopropylacrylamide). Because the release process does not require enzymatic digestion, the ECM is preserved. In SWAT, the preserved ASC ECM serves two functions: (1) it recapitulates the native stromal environment and (2) it rapidly and powerfully bonds the ASC sheets, thus overcoming WAT buoyancy.

Unlike other adipose MPS,[33,34] SWAT utilizes primary human WAT rather than diffAds or rodent adipocytes. This distinction matters because diffAds fail to recapitulate primary adipocyte physiology even under optimized conditions.[13,15–17] For example, we previously showed that human pluripotent cells fully differentiated into diffAds express key adipocyte genes at <1% of primary WAT, even under lentiviral-driven conditions.[13] Soukas *et al.* further showed that *in vitro* and *in vivo* differentiation is transcriptionally distinct, suggesting that diffAds are an incomplete model

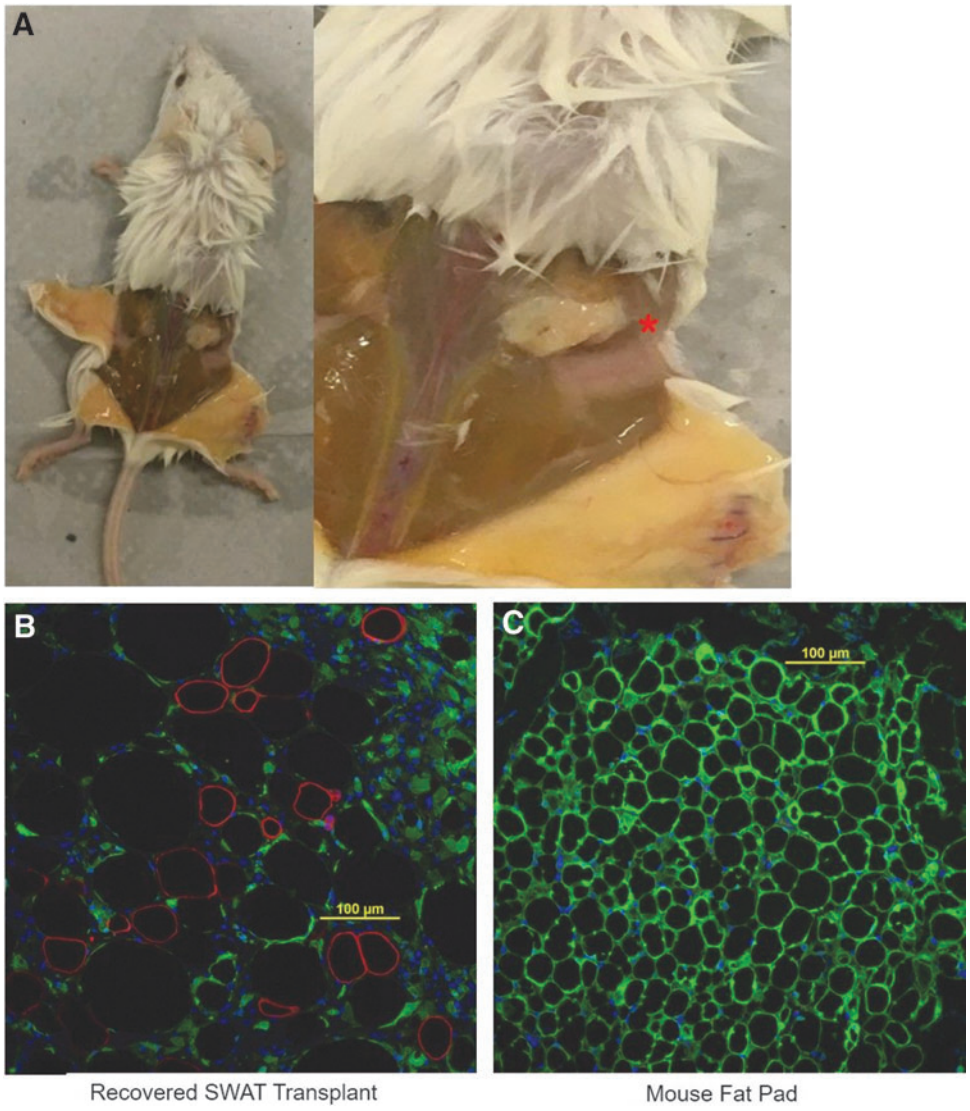

FIG. 6. SWAT can be reimplanted *in vivo*, demonstrating preserved whole-tissue function. **(A)** SWAT was readily visualized 10 days after transplantation into an immunocompromised mouse (*asterisk*). Had SWAT not successfully implanted, by 10 days the necrotic SWAT would have been liquefied. **(B)** Sections of recovered SWAT demonstrated large unilocular adipocytes (diameters 50–120 μm) that were $eGFP^{-}$. Of the total adipocytes, 31.3% were human $PLIN^{+}$. The human $PLIN^{+}$ adipocytes tended to be smaller, which would fit with clinical observations of fat grafting in humans where smaller adipocytes are more likely to survive the transfer. Scale bar = 100 μm, 200× magnification. **(C)** Contralateral fat pads taken from the same mice demonstrated much smaller adipocytes (20–40 μm) and were human $PLIN^{-}$. Scale bar = 100 μm, 200× magnification.

of adipogenesis.[15] Similarly, it has long been recognized that the physiologies of rodent and human adipocytes differ in crucial aspects, such as the density of beta-3-adrenoceptors.[9,11] Because of these shortcomings, it is crucial that adipose MPS utilizes primary human WAT.

For adipose biologists, SWAT offers several advantages. First, the ability to culture SWAT for weeks allows the study of slow-developing WAT phenomena such as adipocyte turnover, hypertrophy, and fibrosis. Second, the non-floating nature of SWAT allows single adipocytes to be monitored over time through previously inapplicable techniques such as time-lapse imaging. Third, the thermo-responsive substrates we used are commercially available and inexpensive, thus avoiding the need for microchip fabrication expertise. Finally, SWAT is readily generated from multiple adipose tissue depots, from both female and male subjects, from normal weight, overweight, and obese subjects, and from diabetic and nondiabetic subjects. These advantages make SWAT a low-cost and highly feasible technique that avoids the need for lengthy differentiation protocols.

Optimization of culture conditions is a primary concern as adipocytes are acutely affected by short-term changes in the fed or starved state *in vivo*. Maintenance of basal gene expression may necessitate regular cycling of satiety hormones, such as insulin and glucagon. Furthermore, current limitations include the absence of a perfusion network, uncertainty regarding the impact of heterologous ASC sources, and the challenges of interperson WAT variability.

SWAT is a new technology and our initial culture conditions do not fully preserve native adipocyte gene expression (Fig. 3). We hypothesize that factors important for fully physiologic adipocyte function are missing, and we will work to identify them in future studies. Many MPS designs include perfusion to model how cells are impacted by microfluidic forces and to provide for future connectivity between multiple model organs.[22] While SWAT does not yet include perfusion, the system is grown on standard culturing plastic, and so we believe it has the capacity to adapt to existing perfusable MPS chip designs.

In this study, the ASC sheets and the WAT used to construct each SWAT were derived from different patients.

The impact of this heterologous construct is unclear and should be the subject of future work. However, in the future it may be possible to generate ''autologous'' SWAT from a single person, particularly for patients undergoing multiple stage procedures (e.g., autologous breast reconstruction). The production of single-source SWAT may offer advantages for personalized medicine, such as pharmaceutical screening and gene therapy.

In summary, the work described in this study validates SWAT as the first primary human WAT MPS. We were able to generate SWAT from healthy subjects, as well as diabetic and obese subjects (disease states). We anticipate that SWAT can generate *in vitro* models for visceral WAT, bone marrow adipose tissue, and brown adipose tissue. In addition, we expect that SWAT will successfully model diseases of human fat such as HIV lipodystrophy and lipomatous tumors. These attributes mark our method as a powerful tool with a wide range of applications in fundamental biological investigations and pharmaceutical development.

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Disclosure Statement

No competing financial interests exist.

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