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Adipose-Derived Stem Cell to Epithelial Stem Cell Transdifferentiation: A Mechanism to Potentially Improve Understanding of Fat Grafting's Impact on Skin Rejuvenation

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Abstract

Background: Recent evidence suggests that lipofilling improves overlying skin composition and appearance. Adipose-derived stem cells (ADSC) have been implicated.

Objective: The authors identify ADSC transdifferentiation into epithelial stem cells through coexpression of GFP+ (green fluorescent protein positive) ADSC with the epithelial stem cell marker p63 in an in vivo fat grafting model.

Methods: Six male, GFP+ mice served as adipose tissue donors. Twelve nude mice served as recipients. Recipients were subdivided into 2 arms (6 mice/each arm) and received either whole-fat specimen (group 1) or isolated and purified ADSC + peptide hydrogel carrier (group 2) engrafted into a 1-cm^2 left parascapular subdermal plane. The right parascapular subdermal plane served as control. Skin flaps were harvested at 8 weeks and subjected to (1) confocal fluorescent microscopy and (2) reverse transcriptase polymerase chain reaction (RT-PCR) for p63 mRNA expression levels.

Results: Gross examination of skin flaps demonstrated subjectively increased dermal vessel presence surrounding whole-fat and ADSC specimens. The GFP+ cells were seen within overlying dermal architecture after engraftment and were found to coexpress p63. Significantly increased levels of p63 expression were found in the ADSC + hydrogel skin flaps.

Conclusions: We offer suggestive evidence that GFP+ ADSC are found within the dermis 8 weeks after engraftment and coexpress the epithelial stem cell marker p63, indicating that ADSC *may* transdifferentiate into epithelial stem cells after fat grafting. These findings complement current understanding of how fat grafts may rejuvenate overlying skin.

Keywords

ADSC, adipose-derived stem cell, epithelial stem cell, p63, fat grafting, skin rejuvenation

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A pluripotential cell line exists within the stromal vascular fraction of adipose tissue known as preadipocytes, or adipose-derived stem cells (ADSC).[1,2] Ease of ADSC harvest through liposuction has facilitated acquisition of stem cell–rich tissue for use in tissue regenerative research, with lipoaspirate demonstrating far more ADSC colony-forming units (CFU) per gram of tissue than bone marrow (5000 vs 100-1000 cells).[3] This equates to an approximately 40 × greater cellular yield from processed lipoaspirate compared with bone marrow aspirate.[3]

With the advent of Illouz's liposuction technique in the 1980s, plastic surgeons obtained access to large volumes of adipose tissue that was routinely discarded. Innovation led to its use for soft-tissue augmentation.[4] Miller is cred-

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ited with introducing the idea of body contour correction by injecting fat parcels through metal cannulas.[5] Coleman[6] offered significant insights into a fat tissue refinement and implantation technique to improve viability and sustainability of clinical results.

While contour restoration remains the primary objective of fat grafting, plastic surgeons have identified additional attributes that grafted fat provides. The quality of skin overlying grafted fat anecdotally demonstrates softened wrinkles, reduced pore size, and improved pigmentation.[7] Others have stated that the skin appears more hydrated and elastic.[8] Grafting under depressed facial scars has been shown to soften and even completely eliminate the scar.[6,7]

Mechanisms to explain the therapeutic effect of ADSC vary. Aged skin models, basic science study, and clinical reviews of fat injection therapy for radiation dermatitis/necrosis have demonstrated that ADSC increase dermal collagen synthesis and vascularity through production of growth factors after implantation.[8-16] Others suggest that ADSC possess the ability to differentiate into epithelial cells.[17-19] While the findings of these latter two studies are noteworthy, they focus on colocalization of ADSC with only 2 of over 19 cytokeratin epithelial cell markers. We consider it appropriate to question the sustainability of these results, as the epithelial skin surface is in a constant state of cellular turnover. Finding ADSC transdifferentiation into cells possessing a marker for epithelial stem cells (ie, p63) may provide greater insight into the mechanism of the lasting skin improvements previously reported after fat grafting and propagate investigation of ADSC within aesthetic and reconstructive skin tissue engineering applications. p63 is a well-documented epithelial stem cell marker that maintains epithelial stem cell proliferative capacity, serves as a significant player in epithelial cell layering, and demonstrates high levels of expression in the progenitor cell layers of skin, breast, and prostate.[20-24]

Our specific aim was to demonstrate ADSC differentiation into skin cell lineages possessing p63 after engraftment in the subdermal plane. To our knowledge, no study has attempted to identify the transdifferentiation of ADSC toward skin cell lineages identified by the p63 stem cell marker. In the present study, we tested PuraMatrix peptide hydrogel (BD Biosciences, Bedford, Massachusetts) as a cell carrier to promote viability of ADSC cells after implantation. The peptide matrix contains amino acids that self-assemble under physiologic conditions to form a fibrous 3-dimensional hydrogel structure that supports cellular attachment. It was originally developed for use in a variety of cell culture experiments.

We hypothesized that by isolating ADSC from the inguinal fat pad of green fluorescent protein positive (GFP+) mice, we would be able to inject them subdermally into a nude mouse and histologically identify ADSC fluorescence in the dermal layer of recipient skin specimens. GFP+ mice have been developed to express GFP in all tissues examined. GFP survives freezing and standard histologic fixation techniques, is intrinsically fluorescent, and represents an ideal reagent for transdifferentiation studies.[25] We anticipated that GFP+ ADSC would coexpress a cellular marker specific for epithelial cells (p63), indicating that injected ADSC may have the potential to transdifferentiate into skin stem cell lineages.

METHODS

Animal Groups and Rationale for Study Population Number

Twelve male C57BL/6 mice (25-30 g) served as the adipose tissue donor population (Jackson Labs, Bar Harbor, Maine). They specifically expressed GFP in all tissues examined. Cell fluorescence aided in histologic identification of grafted specimens from this donor group. Twelve nude B6.Cg-Foxn1^nu mice served as the recipient population (Jackson Labs). Both mice populations were 14 weeks old at the time of delivery to our laboratory and appropriately acclimated for 2 weeks prior to experimental induction. Nude mice were subdivided into 2 arms (6 mice/arm). Experimental arms received whole inguinal adipose specimen (group 1) or ADSC + glucose + peptide hydrogel (group 2) grafted respectively into the left parascapular subdermal plane. The right parascapular subdermal plane was subjected to 1 of 3 control parameters—0.2 mL of vehicle peptide hydrogel subdermal injection, phosphate-buffered saline (PBS) subdermal injection, or sham surgery with a subdermal pocket dissected for 1 cm^2—followed by simple skin closure. Our study population number was comparable to that of published studies with a similar design.[26]

Fat Graft Harvest

Fat was excised from the C57BL/6 mice in accordance with a protocol (#158-10-005) approved by the Laboratory Animal Care and Use Committee at Southern Illinois

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University School of Medicine. Briefly, after induction with isofluorane (1%-3%), adequacy of anesthesia was confirmed and the GFP+ mice were laid supine. Each mouse's abdominal wall and groin were prepped with 4% chlorhexidine gluconate topical antiseptic solution (Aplicare, Inc, Meriden, Connecticut). Incisions were made obliquely along the inferoanterior abdominal wall. Abdominoinguinal fat pads were isolated, excised, rinsed with PBS, and transferred to sterile tubes containing Dulbecco's modified Eagle's medium (DMEM), stored on ice for 3 minutes during transfer to a sterile laboratory hood for tissue processing, or promptly transferred to the experimental/left side as fresh tissue in whole-fat graft (group 1) nude mice recipients.

Isolation of GFP+ ADSC From Mice Adipose Tissue and Amplification In Vitro

Adipose tissue was aseptically transferred to sterile tissue culture dishes in a laminar flow hood and mechanically dissociated. Dissociated tissues were incubated in a 33% antibiotic/antimycotic solution for 30 minutes at 4°C to inhibit potential contamination. The tissue then was washed with PBS and digested with collagenase for 40 minutes at 37°C in a shaking water bath. Collagenase was neutralized with growth media containing 10% fetal bovine serum (FBS); single cells were isolated by filtering the suspension through a 70-µm nylon mesh strainer (BD Falcon–BD Biosciences, Bedford, Massachusetts). Stromal vascular fraction (SVF) was pelleted by centrifugation at 1000 rpm for 3 minutes. Red blood cells (RBC) were lysed with red blood cell buffer (Sigma, St. Louis, MO), and SVF was recentrifuged at 1000 rpm for 3 minutes and pelleted. SVF was then plated for expansion in growth media (DMEM, 10% FBS, 1% penicillin/streptomycin, 4 mM L-glutamine). Expanded SVF was harvested and subjected to flow cytometry (fluorescence-activated cell sorting [FACS]) to characterize and ensure phenotype as set forth by the International Society for Cellular Therapy.[27] This ensured accuracy and consistency of established definitions of ADSC within this project. FACS was performed once. Cells were washed with PBS and incubated with monoclonal antibodies (mAbs) for 30 minutes at 4°C. mAbs included Alexa Fluor 647–conjugated CD34 mAb (BD Pharmingen–BD Biosciences, San Jose, California), V450-conjugated CD73 mAb (BD Horizon–BD Biosciences, San Jose, California), and PE/Cy7-conjugated CD105 mAb (BioLegend, San Diego, California). In our laboratory's experience, 1% to 3% of processed SVF has yielded a CD34+ nucleated cellular fraction, comparable to other published data.[3] Cells were subsequently expanded for 3 passages (corresponding to approximately 3 population doublings per passage) in growth media (DMEM, 10% FBS, 1% penicillin/streptomycin, 4 mM L-glutamine) prior to further use. The ADSC populations were CD34+, CD105+, CD73+, CD31–, CD45–, CK19–, and P63– at passage 3 as determined by immunofluorescent staining. In vivo ADSC engraftment experiments were performed with cells at passages 2 to 4. Note that p63 was not present in our cell samples prior to injection.

Preparation of BD PuraMatrix Peptide Hydrogel

To prepare a cell carrier from peptide hydrogel, 1% stock solution was mixed with 20% sucrose (Sigma-Aldrich Sweden AB, Stockholm, Sweden) and DMEM containing Ca2+ and Mg2+ in proportion 2:1:1, which resulted in 0.5% hydrogel. To prepare hydrogel containing cultured cells, ADSC were detached from the culture flasks with trypsin/EDTA, washed, and assessed for viability with trypan blue (Hyclone Labs, Logan, Utah). The cells were concentrated in their own growth medium, resuspended in 20% sucrose to prevent gel formation, and added to the hydrogel so that after mixing with the hydrogel, a final concentration of 1×10^6 cells/injection site was obtained. The solution was left for 1 minute and mixed gently with pipette, and the resulting hydrogel was used for experiments.

Graft and ADSC + Peptide Hydrogel Implantation

Nude mice were recipients of GFP+ whole-fat (group 1) or peptide hydrogel + ADSC injectate (group 2) samples (Figure 1). For the whole-fat group (group 1), the experimental left parascapular region was marked and 1-cm^2 skin flaps were elevated, incised only along the proximal margin. A 0.25-g to 0.5-g donor whole-fat graft was placed in the left parascapular pocket and skin was closed using a 6-0 Prolene suture (Ethicon, Inc, Somerville, New Jersey) in simple interrupted fashion. For peptide hydrogel + ADSC injectate (group 2), 200 µL of ice-cold B PuraMatrix peptide hydrogel with 1×10^6 ADSC were injected subcutaneously into a left parascapular subdermal plane using a 1-mL insulin syringe with a 23-gauge needle. Injections were performed slowly, allowing peptide hydrogel to polymerize and form a jelly-like implant with an irregular shape under the skin. The right parascapular subdermal plane served as control (PBS or peptide hydrogel injection or sham). After whole-fat or ADSC implantation, mice were reversed from general anesthesia with supplemental 100% O_2 support. Animals were observed for 15 minutes postoperatively and then placed in individual cages to resume activity ad libitum. Postoperatively, experimental animals were cared for in an air-filtered barrier facility and received autoclaved nutrition in a sterile working environment. Mice were housed 1 per cage postoperatively, fed

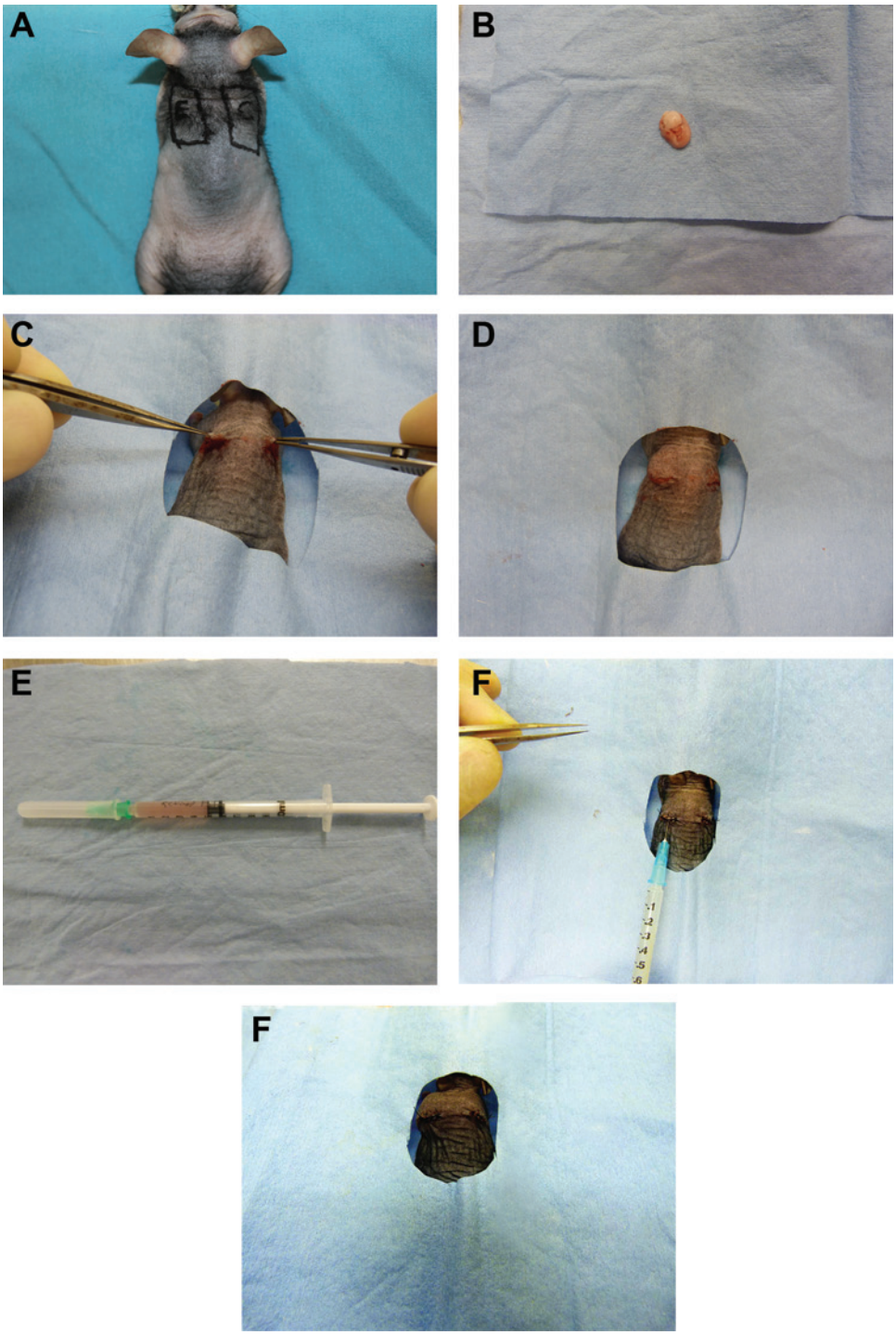

Figure 1. Nude mouse (a) in preparation for whole-fat graft (group 1 [b-d]) or adipose-derived stem cells (ADSC) + peptide hydrogel (group 2 [e-g]) engraftment. Experimental left-sided parascapular skin flaps were elevated and received fat engraftment via the described techniques. Right-sided parascapular skin flaps were elevated and subjected to 1 of 3 control parameters (hydrogel, phosphate-buffered saline, or sham surgery).

chow ad libitum, and subjected to physical examinations daily.

Tissue Harvest and Processing

At 8 weeks, recipient mice were sacrificed using inhalational anesthetic overdose. Skin flaps from experimental and control sides were excised sharply, divided, and then fixed in either cold 4% paraformaldehyde (pH 7.4) for histologic assay or frozen in Allprotect (Qiagen, Valencia, California) for RNA and protein extraction. For skin tissue preparation, specimens were fixed in 10% neutral buffered formalin overnight, embedded in OCT compound (Sakura, Torrance, California), frozen in a dry ice/ethanol bath, and stored in −70°C until use. Specimens were then cross-sectioned (10-micron-thick) for further histologic assays and immunofluorescent staining.

Fluorescence Observation of Skin Specimens and Standard Histology

Immunohistochemical analysis was performed to identify engrafted whole-fat and hydrogel + ADSC on the basis of GFP signal and to identify the phenotype of engrafted stem cells with specific antibodies against additional antigens. Primary antibodies against the following epitopes were used in this series: p63 (ab53039; rabbit–anti-mouse; 1:500) (Abcam, Cambridge, Massachusetts), Ki67 (ab15580; rabbit–anti-mouse; 1:50) (Novus Biologicals, Littleton, Colorado), and cytokeratin 19 (ab15463; cytokeratin 19; 1:200) (Santa Cruz Biotechnology, Santa Cruz, California). Prior to antibody staining, slides were washed in PBS and fixed with 4% paraformaldehyde for 10 minutes at room temperature. Next, slides were washed with PBS containing 0.3% Triton X-100 (Sigma-Aldrich, St Louis, MO) and subjected to blocking solution of 1% bovine serum albumin (BSA) for 60 minutes at room temperature. Primary antibody incubation followed overnight at 4°C, followed by PBS wash and incubation with Texas Red Goat Anti-Rabbit IgG secondary antibody (Vector Labs, Burlingame, California). Slides were again washed and mounted by Prolong Gold with DAPI (Invitrogen, Grand Island, New York) with coverslips. Slides were analyzed and photomicrographs taken using a Leica confocal microscope (Leica Microsystems, Buffalo Grove, Illinois). Standard histology assays were performed by hematoxylin and eosin (H&E) staining.

Statistical Analysis

Group mean values differences, for p63 expression, were determined using the Student *t* test ($*P < .05$; $**P < .01$).

RESULTS

Flow Cytometric Characterization and Purification of ADSC and Immunophenotype Characterization of Isolated GFP+ ADSC

Flow cytometry data confirmed isolation of the ADSC cellular fraction that expressed cell surface markers CD34 + CD73 + and CD105 + . This expression profile is normal for mesenchymal stem cells (MSC) according to the International Society for Cellular Therapy statement of minimal criteria for defining MSC.[27] Representative fluorescent imaging of isolated ADSC demonstrates the presence of the ADSC cell surface marker CD73 in our ADSC population (Figure 2). CD34, an adipose MSC surface marker, while initially present in our ADSC isolate, was lost during the 3-passage process (data not shown). To ensure that the ADSC isolate was pure and not contaminated with other cellular fractions, we confirmed the absence of cell surface markers CD31 (endothelial), CD45 (white blood cell), p63 (epithelial stem cell marker), and CK19 (cytokeratin) by immunofluorescence staining. Fundamental to this investigation, we noted that p63 was *not* present in our sample prior to injection (Figure 2).

Gross Morphology and Angiogenic Effect of Engrafted Whole Fat and ADSC + Peptide Hydrogel

Our results were consistent with well-documented effects of fat grafting's impact on surrounding, local tissue after fat engraftment.[8-16] Increased angiogenesis, or vessel growth from existing vasculature, was noted on the dermal side of experimental skin flaps at an 8-week harvest for groups 1 and 2 (Figure 3). Similar findings were not noted on control skin flap specimens. At week 1, peptide hydrogel was seen adherent to, and well demarcated from, the dermal side of elevated skin flaps. One-week skin protein expression analysis was not performed in this study but is part of forthcoming analyses. By 8 weeks, the hydrogel had grossly resorbed, suggesting biocompatibility with local tissue (Figure 3).

Peptide Hydrogel Permits ADSC Survival After Engraftment In Vivo

High-powered fluorescent merged representative microscopic images for group 2 demonstrate effective cellular encapsulation of ADSC by the peptide hydrogel. This offered evidence of ADSC viability within the hydrogel scaffold prior to further investigation of cellular migration patterns (Figure 5).

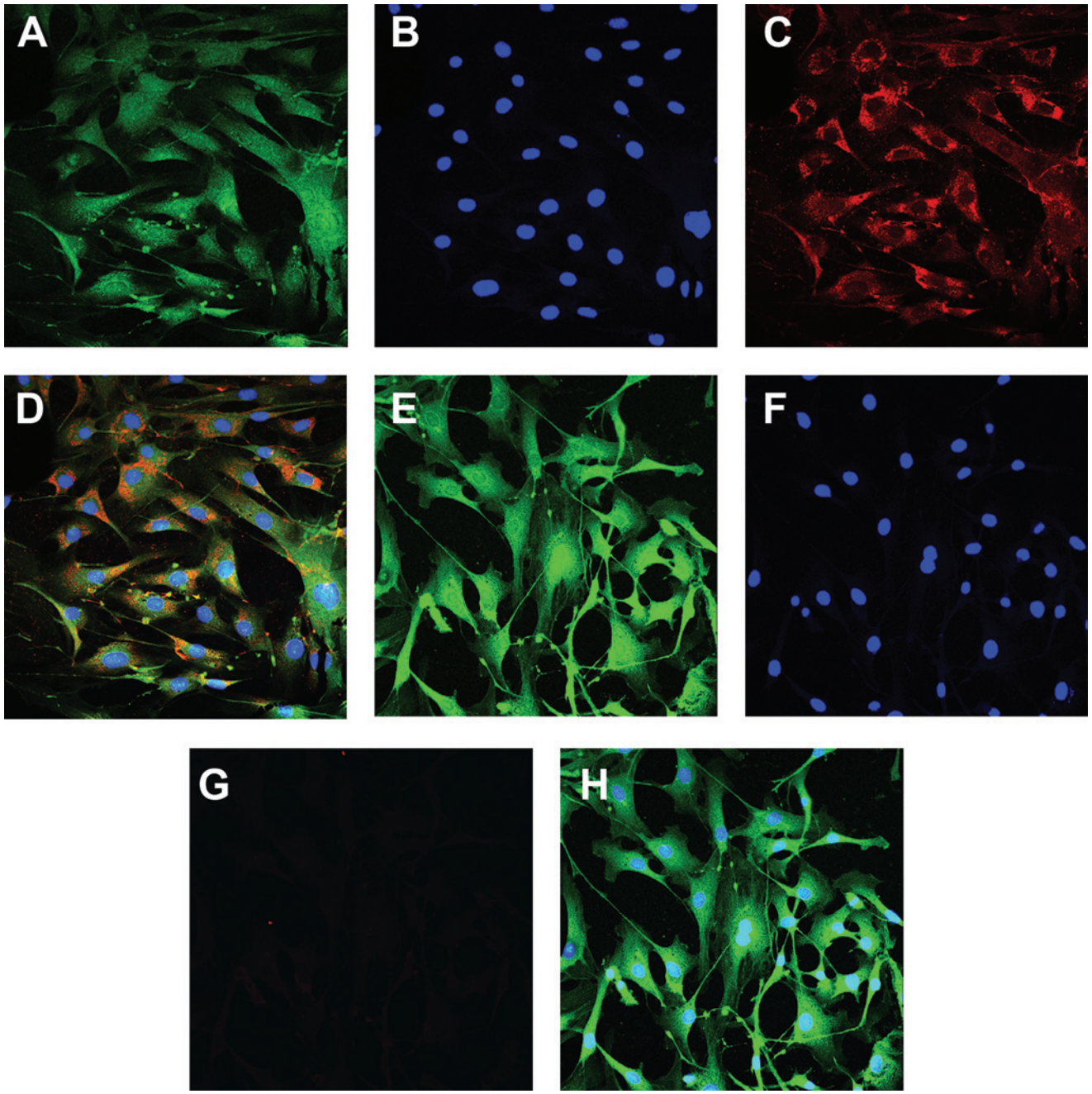

Figure 2. Adipose-derived stem cell (ADSC) cellular isolation and purification. Isolation of CD34 + /CD73 + /CD105 + ADSC was performed through fluorescence-activated cell sorting analysis. Images of our green fluorescent protein positive (GFP +) ADSC (A), their blue nuclei (B), and the red cell surface marker of interest (CD73 +) (C) are merged to demonstrate the brown color of CD73 coexpression with our GFP + ADSC (D). This process was repeated for CD34 and CD105. To confirm the absence of p63 within our ADSC sample prior to engraftment, note the merged image (H) that combines images E-G. This process was performed also to confirm ADSC sample purity, as our sample tested negative for endothelial cells (CD31–), white blood cells (CD45–), and epithelial cells (CK19–).

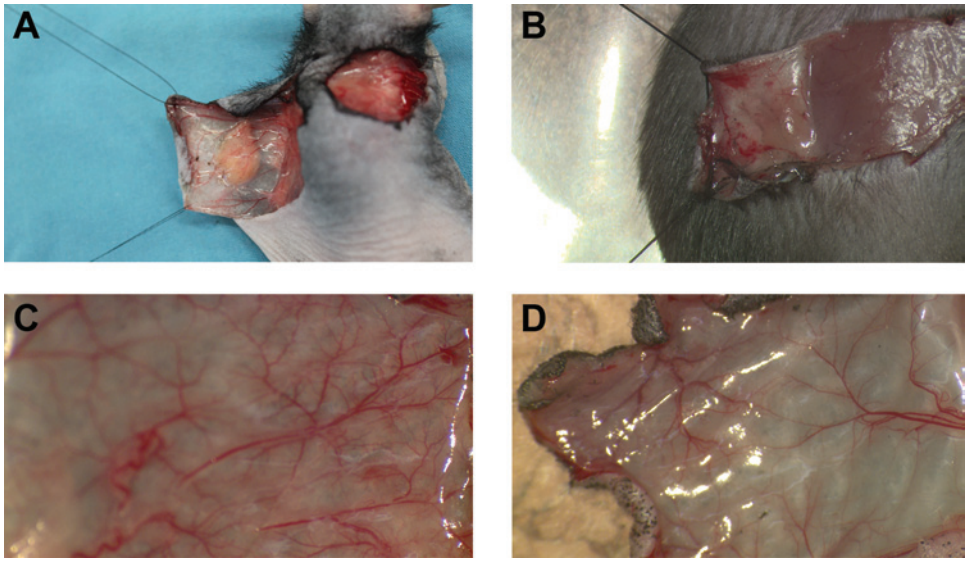

Figure 3. Angiogenesis on the dermal side of experimental skin flaps seen in both whole-fat (A) and adipose-derived stem cells (ADSC) + hydrogel (B) experimental groups. Resorption of hydrogel is seen between 1 and 8 weeks after engraftment (C, D).

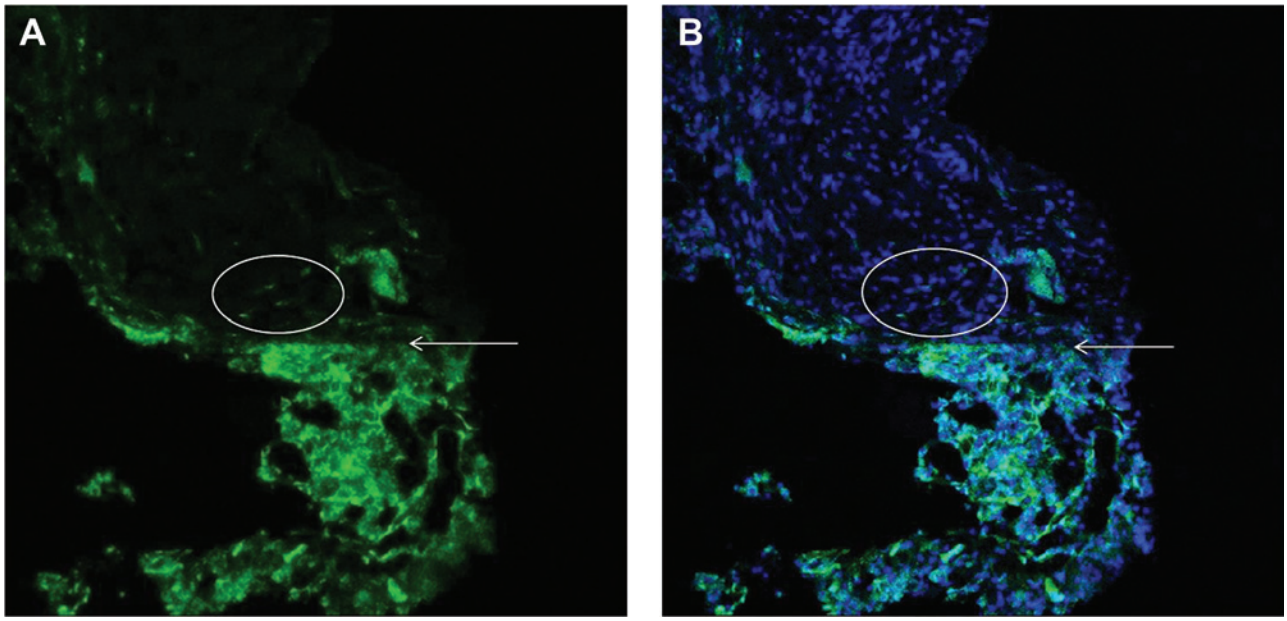

Figure 4. Tracking donor green fluorescent protein (GFP)–producing cells after whole-fat engraftment (group 1) (A). Arrow denotes the dermal-hypodermal junction and circle indicates skin architecture region of interest containing GFP + cells. Merged imaged confirms presence of GFP + coexpression with the blue nuclear DAPI signal signifying a cellular GFP + presence within overlying dermis at 8 weeks after engraftment (B). Original magnification: × 40.

GFP+ Cellular Presence Within Dermal and Epidermal Architecture and Transdifferentiation Potential of Engrafted ADSC

Whole-fat graft images demonstrated clear delineation between the GFP + whole-fat graft and overlying skin. GFP + cells were seen within overlying dermal architecture (Figure 4). Group 2 confocal images confirm viability of GFP + ADSC within the hydrogel scaffold at 8 weeks (Figure 5). The fluorescence of the GFP + C57BL/6 donor mice ADSC population we used "remains constant until cell death" and is readily identified by FACS or fluorescent microscopy.[25]

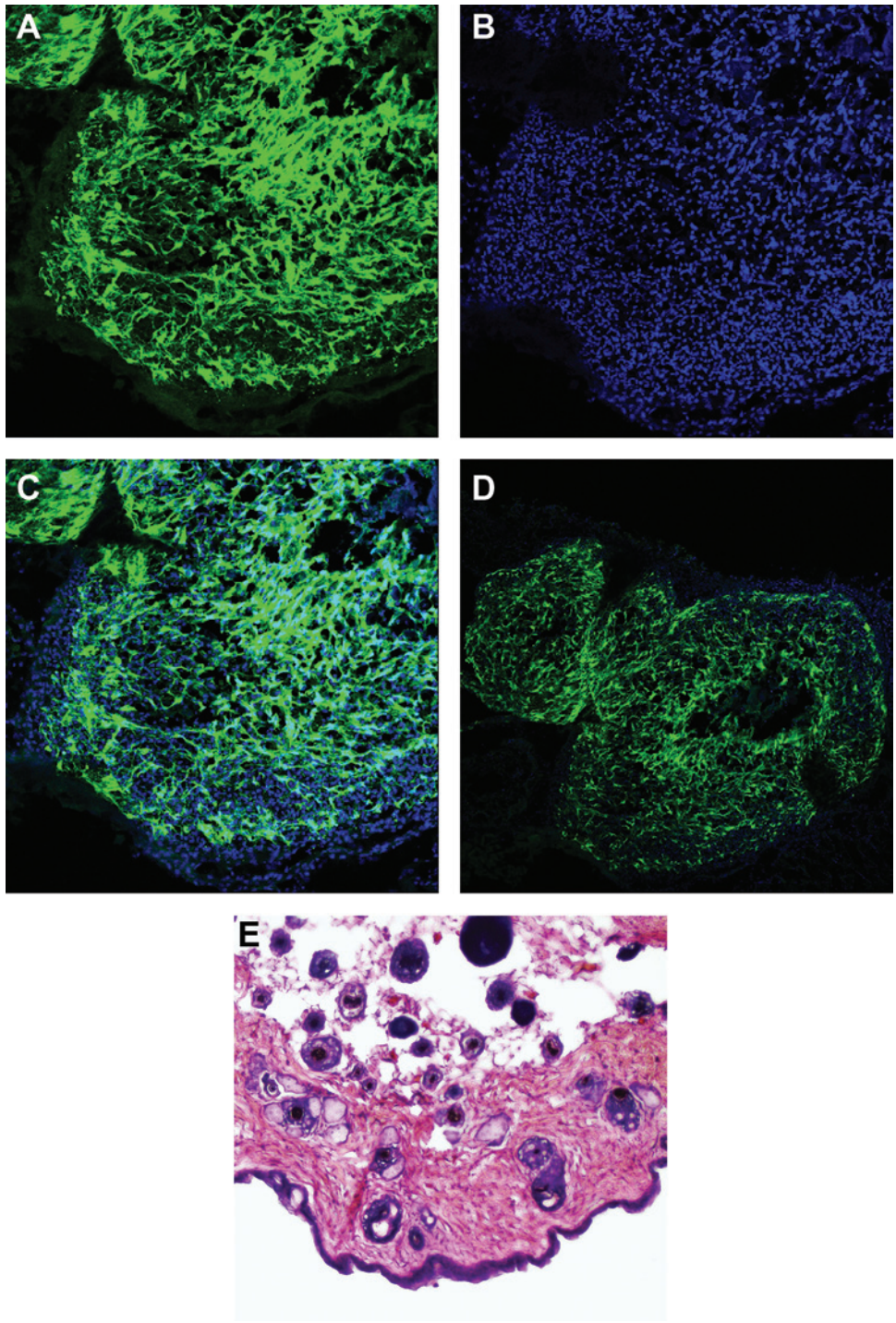

Figure 5. Confirmation of adipose-derived stem cell (ADSC) isolate cellular viability at 8 weeks (group 2). Merged images (A + B) on the left (C) confirm green fluorescent protein positive (GFP+) coexpression with DAPI (nuclei), signifying cellular presence in the hypodermal region at 8 weeks in the ADSC + hydrogel group. Note how the hydrogel served to keep ADSC local to the overlying dermis after engraftment (D). Standard hematoxylin and eosin stain for orientation (E). Lower power image magnification: ×40. High-power image magnification: ×100.

Notably, and sentinel to this study, high-powered, merged (GFP+ p63) fluorescent images demonstrated that ADSC are found within overlying skin and are concomitantly able to express the epithelial stem cell marker, p63, at 8 weeks after engraftment (Figure 6). Staining for the nuclear marker of proliferation, Ki67, within engrafted GFP+ ADSC at 1 week suggests ongoing proliferation of the ADSC cell line (Figure 7).

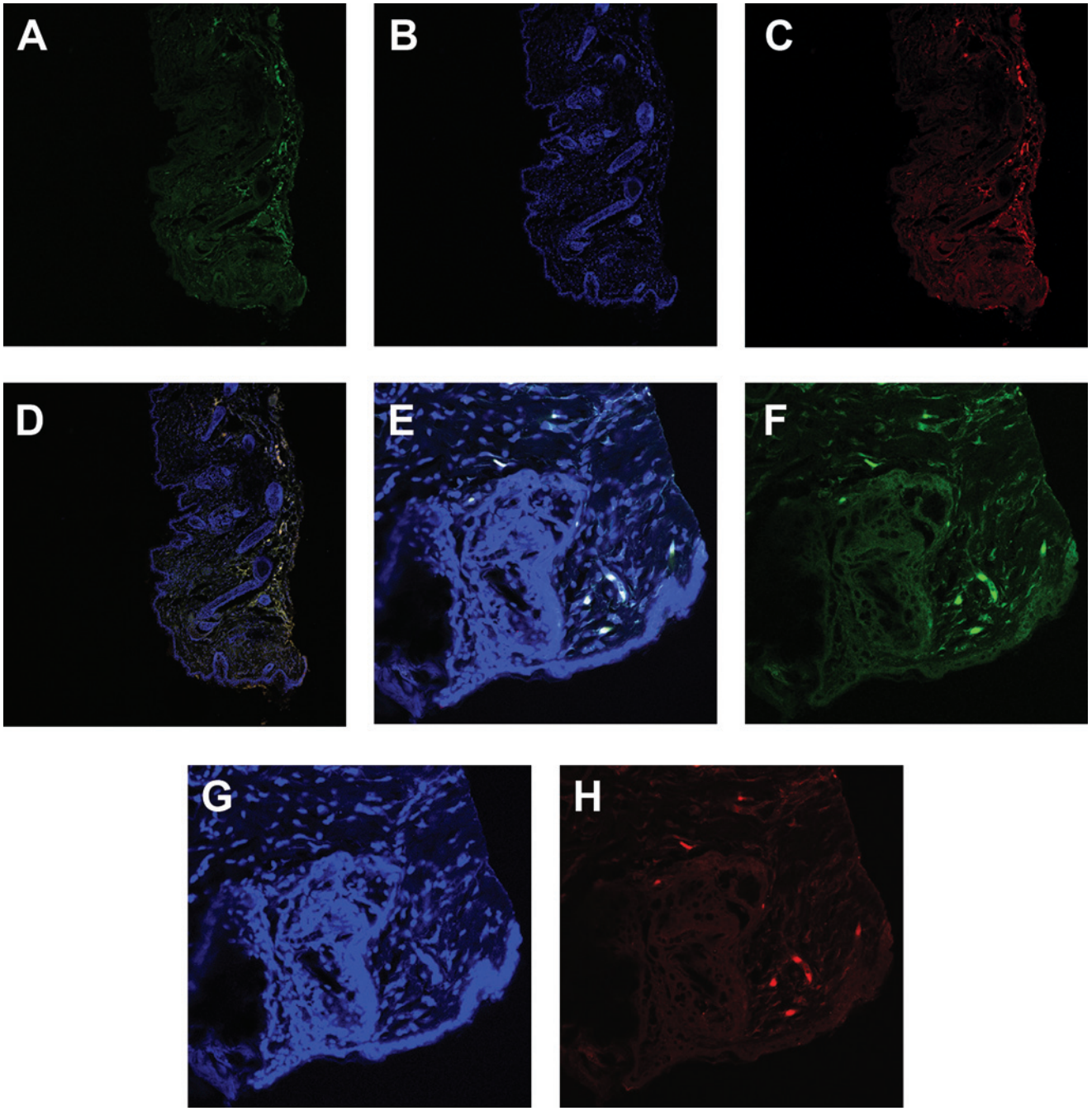

Figure 6. Confocal imaging suggestive of engrafted green fluorescent protein positive (GFP+) adipose-derived stem cells (ADSC) coexpressing the epithelial stem cell marker, p63 (red). Images D and E represent the merging of images A-C and F-H, respectively. Note the light gray/pale brown coexpression of p63 with the nuclei of the ADSC found within the dermis (circles surround dermal area of focus). Original magnification: ×40 (a-d), ×100 (E-H).

In addition to these findings, engrafted GFP+ cells were observed incorporating as components of the regenerated epidermal epithelium at 8 weeks on the basis of GFP cells costaining for the cytokeratin marker of epidermal epithelium cytokeratin 19 (CK19) (image not shown).

DISCUSSION

Current literature cites neovascularization, increased dermal collagen production, and antioxidant effects secondary to ADSC influence as the mechanisms through which fat

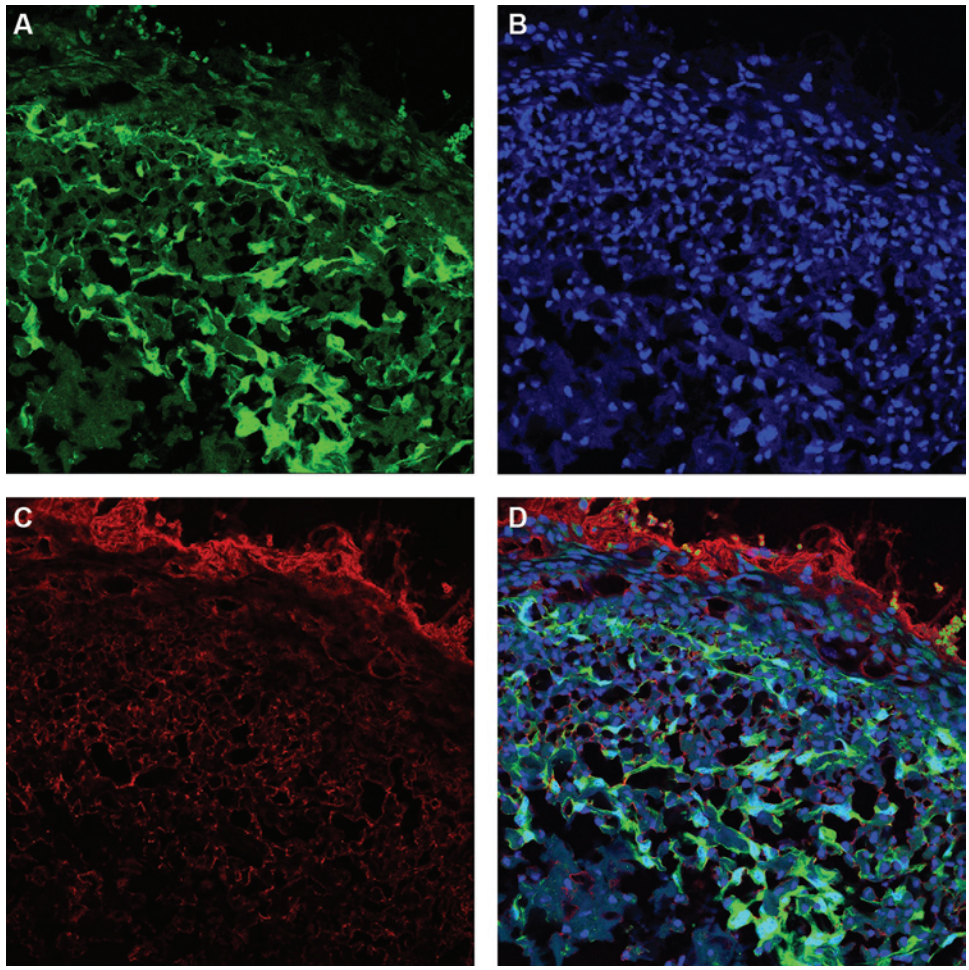

Figure 7. Confocal imaging suggestive of engrafted green fluorescent protein positive (GFP+) adipose-derived stem cells (A) (with their blue nuclei [B]) coexpressing the marker for nuclear replication, Ki67 (C), at 1 week. Merged image (D) demonstrates the pale brown/yellow coexpression of Ki67 with GFP+ cells scattered throughout the epidermis. Note: epidermis is oriented to the top of the merged image. Original magnification: ×100 (all images).

grafts "rejuvenate" overlying skin and facilitate wound healing.[9,11-13,28-32] While these investigations have provided thorough histologic analyses to support these conclusions, we suggest that ADSC may affect skin through mechanisms beyond paracrine effects alone.

Prior research, focused on ADSC to skin cell transdifferentiation, demonstrated the ability of ADSC to express the epithelial cell marker, cytokeratin 19, 4 weeks after scaffold engraftment in a wound-healing model.[18] When, however, we consider that all stratified epidermal structures are in a constant state of cellular turnover, ADSC to keratinocyte differentiation seems less likely to represent a sustainable mechanism for explaining fat grafts' lasting contribution to skin rejuvenation. We postulated that demonstration of ADSC to epithelial stem cell transdifferentiation might stimulate further investigation of this possible mechanism to explain fat grafting's effect upon overlying skin.

We identified that GFP+ cells can be found within overlying skin architecture after GFP+ whole-fat engraftment (Figure 4). We could not, however, accurately assume that these cells from whole fat are definitively ADSC, as multiple cell types exist within the SVF. To minimize this possibility, we isolated, purified, and expanded our ADSC population through flow cytometry based on 3 accepted cell surface markers for adipose stem cell isolate (CD34/73/105).[27] We emphasize that the p63 epithelial stem cell marker was *not* present in our isolated and purified ADSC samples prior to engraftment. Therefore, what are the possible options to explain why we saw p63 coexpression with GFP+ cells at the dermal level? The first possibility is that our ADSC were, in fact, transdifferentiating into epithelial stem cells and in transit to the basal epithelial layer. Second, macrophages could have engulfed our ADSC and migrated into the dermis, where they were stimulated to become epithelial stem cells. A third possibility is that our ADSC differentiated into other cell lines that subsequently differentiated into p63-expressing cells, instead of direct ADSC to epithelial stem cell transdiffer-

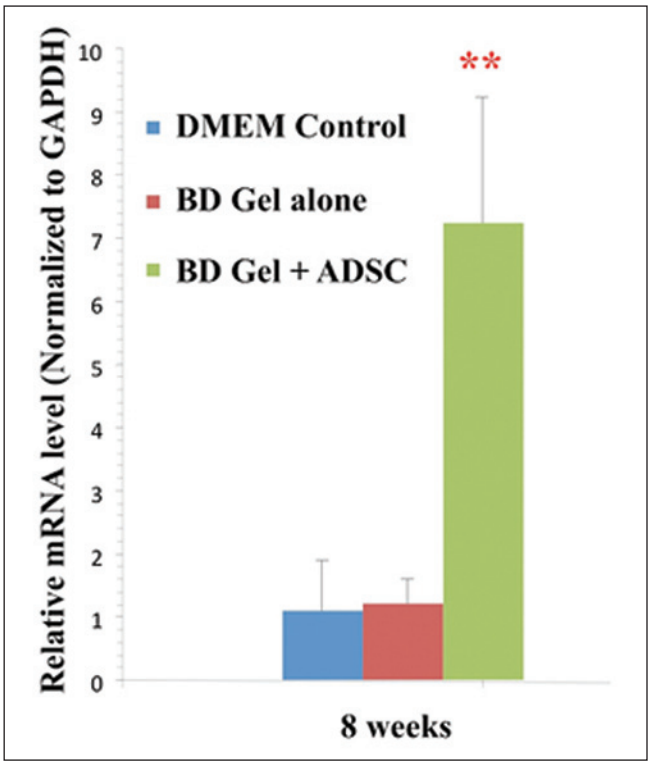

Figure 8. Real-time polymerase chain reaction analysis of p63 expression levels at 8 weeks after fat engraftment. Gene expression levels are significantly increased in skin specimens overlying hydrogel + adipose-derived stem cell (ADSC) engraftment compared with control specimens (Dulbecco's modified Eagle's medium [DMEM] or hydrogel alone). $^{**}P < .01$.

entiation. Of note, our ADSC did coexpress the nuclear marker of proliferation, Ki67, suggestive of ADSC viability at 1 week, rather than phagocytosis and breakdown by resident macrophages within the highly immunogenic subdermal environment after engraftment. Further isolation and analysis of p63 expressing cells within our skin samples, and forthcoming cellular co-culture studies, may lend insight into the cell-cell interactions alluded to previously.

At 8 weeks after engraftment, p63 expression levels were significantly greater in the ADSC + hydrogel group (group 2) skin flaps compared with control specimens (Figure 8). Note that relative messenger RNA (mRNA) expression level of p63 for all group treatments was based on p63 mRNA expression of sham mice. p63 mRNA expression of sham mice is 1. The formula for the calculation of other group expression levels is $2^{[\text{target gene count (treatment group)} - \text{target gene count (sham control group)}]}/2^{[\text{GAPDH count (treatment group)} - \text{GAPDH count (sham control group)}]}$. While the exact cellular source of p63 expression may not be able to be confirmed based on the results of our study, future studies should evaluate long-term p63 expression levels in the basal layers of skin and how suppression and upregulation of p63 may alter skin composition.

By using a carrier to maintain GFP + ADSC cellular consolidation during the engraftment period (ie, peptide hydrogel), we provided evidence that our ADSC injectate remained local to the skin study area of interest throughout the study duration. Our findings demonstrated that peptide hydrogel served as a suitable synthetic extracellular matrix for cultured ADSC, with biocompatibility demonstrated. Hydrogel resorbed by 8 weeks after engraftment in this model (Figure 3). While 1-week postengraftment protein expression analysis was not reported in this study, forthcoming studies from our institution will examine temporal p63 protein expression levels. One-week skin flap gross analysis has been included solely to confirm the hydrogel carrier's persistence and proximity to the overlying dermis after engraftment.

Taken together, our findings allow us to conservatively suggest that ADSC possess the ability to populate the skin architecture after fat engraftment and may possess the ability to transdifferentiate into epithelial stem cells given their coexpression with p63. These findings potentially indicate that ADSC do more than influence overlying skin through paracrine mechanisms alone, but also contribute directly to skin cell lineages through ADSC to epithelial stem cell transdifferentiation. We hope such insight lends itself to further investigational study aimed at determining changes in epithelial stem cell composition of aged skin and the effects that upregulating or downregulating epithelial stem cell composition within skin may have upon the cutaneous aging process.

CONCLUSIONS

Fat grafting holds promise as a tool for skin rejuvenation that complements its well-established utility as an autologous filler. Explanations of the mechanisms through which fat affects overlying skin are broad. ADSC are considered to contribute. We sought to add to current understanding of ADSC physiologic effects upon skin after engraftment. Our observation of ADSC migration into overlying skin through donor cell tracking and GFP + cells costaining with p63 is supportive of the claim that ADSC may be able to transdifferentiate into epithelial stem cells. Correlative upregulation of p63 (an epithelial stem cell marker) after ADSC engraftment offers further support of this claim. Future research may help clarify whether inhibition or upregulation of p63 + epithelial stem cells results in acceleration or deceleration of the skin aging process and may lend insight into the importance, or lack thereof, of our current findings.

Disclosures

The authors declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

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