Differential Contributions of Graft-Derived and Host-Derived Cells in Tissue Regeneration/Remodeling after Fat Grafting

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Background: Recent research indicates that the adipose tissue of nonvascularized grafts is completely remodeled within 3 months, although origins of next-generation cells are unclear.
Methods: Inguinal fat pads of green fluorescent protein mice and wild-type mice were cross-transplanted beneath the scalp. At 1, 2, 4, and 12 weeks after transplantation, grafted fat was harvested, weighed, and analyzed through immunohistochemistry, whole-mount staining, and flow cytometry of cell isolates. Bone marrow of green fluorescent protein mice was transplanted to wild-type mice (after irradiation). Eight weeks later, these mice also received fat grafts, which were analyzed as well.
Results: The majority of host-derived cells detected during remodeling of grafted fat were macrophages (>90 percent at the early stage; 60 percent at 12 weeks). Cell origins were analyzed at 12 weeks (i.e., when completely regenerated). At this point, mature adipocytes were largely derived from adipose-derived stem/stromal cells of grafts. Although vascular wall constituents were chiefly graft derived, vascular endothelial cells originated equally from graft and host bone marrow. Adipose-derived stem/stromal cells of regenerated fat were an admixture of grafted, host nonbone marrow, and host bone marrow cells.
Conclusions: The above findings underscore the importance of adipose stem/stromal cells in the grafted fat for adipocyte regeneration. Host bone marrow and local tissues contributed substantially to capillary networks and provided new adipose-derived stem/stromal cells. An appreciation of mechanisms that are operant in this setting stands to improve clinical outcomes of fat grafting and cell-based therapies. (*Plast. Reconstr. Surg.* 135: 1607, 2015.)

Fat grafting has been increasingly recognized for its many clinical benefits, aside from tissue volumization. Tissues depleted of stem cells may be corrected by stem cell–containing tissue grafts. Grafted fat has the potential to revitalize diseased tissues (irradiated, dystrophic, or ischemic) and painful scars, thanks to contributing adipose-derived stem/stromal cells and tissue. However, such benefits are achieved only when each procedural step of fat grafting is properly executed. Hence, it seems that there remains much room for technical improvement.

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Received for publication September 17, 2014; accepted December 29, 2014.
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DOI: 10.1097/PRS.0000000000001292

Adipose tissue harbors a variety of cells, namely adipocytes, adipose-derived stem/stromal cells, and blood vascular cells (endothelial and mural).[1] Isolated or cultured adipose-derived stem/stromal cells hold great therapeutic promise through their capacity for multilineage differentiation, paracrine activity, and immunomodulation.[2–6]

Disclosure: *The authors have no financial interest to declare in relation to the content of this article.*

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Consequently, clinical investigations of adipose-derived stem/stromal cells are aimed at a multiplicity of disorders[7,8]

Adipose-derived stem/stromal cells are heavily involved in the remodeling of adipose tissue after trauma of any nature, including ischemia-reperfusion injury, mechanical force, and grafting.[9–12] Through previous efforts, we have delineated a range of events that occur during remodeling, particularly the replacement of adipocytes by next-generation cells after fat is grafted.[12,13] Although cells such as adipose-derived stem/stromal cells, vascular endothelial cells, and macrophages are known participants in remodeling,[13,14] the origins of these constituents are unclear. It is acknowledged that adipose-derived stem/stromal cells facilitate mobilization and homing of bone marrow cells (such as endothelial progenitor cells)[15]; therefore, bone marrow—derived cells are likely components of regenerating fat after grafting.

In this study, we sought to investigate the origin of cells in the grafted fat after tissue remodeling/regeneration. We removed fat from green fluorescent protein (GFP) and wild-type mice for cross-transplantation (i.e., fat exchange) to investigate the origins of regenerating cells thereafter. In addition, mice with GFP–positive bone marrow served as recipients to research the fate of bone marrow–derived cells. We believe that a better appreciation of mechanisms inherent in the remodeling of grafted fat will improve related clinical outcomes and broaden the potential of stem cell therapies.

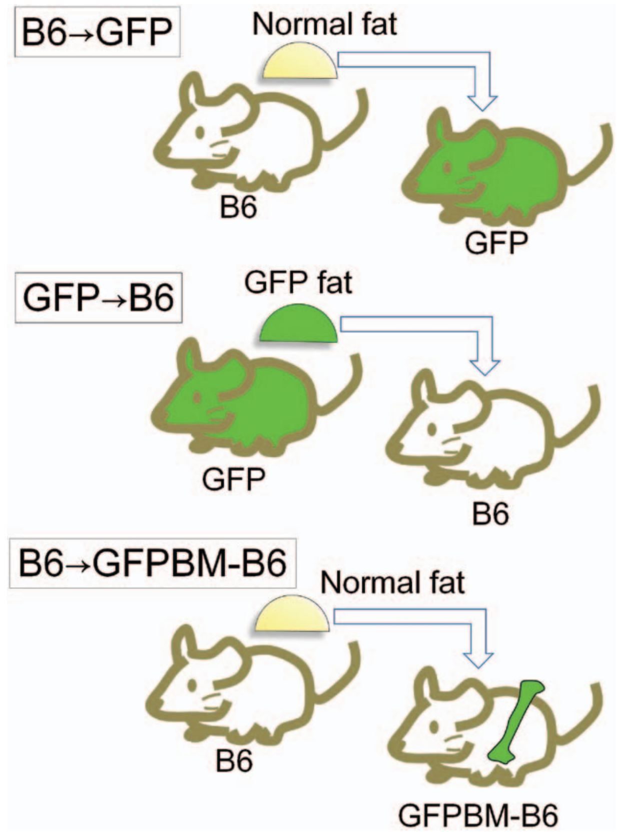

Fig. 1. Schematic diagram of experimental animal models. The inguinal fat pads of B6 mice were transplanted to GFP mice (B6→GFP), and those of GFP mice were transplanted to B6 mice (GFP→B6). GFP-positive bone marrow chimeric B6 mice (*GFPBM-B6*) were prepared by transplanting bone marrow of GFP mice into irradiated B6 mice. The fat pads of B6 mice were also transplanted to GFPBM-B6 mice (B6→GFPBM-B6).

MATERIALS AND METHODS

Animal Models

All animals were obtained from Japan SLC (Shizuoka, Japan; http://www.jslc.co.jp). Animal maintenance and experimental protocols were conducted under University of Tokyo guidelines.

Fat Exchange Models

Female 9-week-old mice of two strains, C57BL/6 (B6 mice) and C57BL/6-Tg (CAG-EGFP) (GFP mice), were anesthetized using intraperitoneal pentobarbital 50 mg/kg, and inguinal fat pads were harvested for cross-transplantation into subcutis of scalp, as described previously.[12] Fat pads of B6 mice were transplanted to GFP mice (B6→GFP), and fat pads of GFP mice were transplanted to B6 mice (GFP→B6) (Fig. 1). B6 and GFP mice are near-identical strains, so immune reactivity is negligible. At 1, 2, 4, and 12 weeks after transplantation, total body weight was recorded, and the grafted fat was harvested for further analyses, such as histology and flow cytometry. Thus, different animals were analyzed at each time point. Three animals for each group at each time point were analyzed for weight, histology, and stromal vascular fraction culture. Furthermore, nine animals for each group at 12 weeks were analyzed for fluorescence-activated cell sorting analysis of stromal vascular fraction. Bodily weight increases over time were similar in both grafted models. Graft weights were normalized (i.e., divided by body weights) to counter the effects of animal growth.

Bone Marrow Transplantation Model

GFP-positive bone marrow chimeric B6 mice (GFPBM-B6) were generated as reported previously.[16] Briefly, femoral and tibial bone marrow cells were harvested from 6-week-old female GFP mice by flushing with phosphate-buffered saline. After hemolysis with red blood cell lysis buffer (Sigma Aldrich, St. Louis, Mo.) and washing,

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unfractionated GFP-positive bone marrow cells (2×10^6 cells/mice) were injected intravenously via tail veins into 6-week-old female B6 mice after lethal doses (10 Gy) of whole-body γ-irradiation. At week 6 after bone marrow transplantation, viable GFP-positive bone marrow chimeric cells were confirmed by flow cytometry of peripheral blood. Only mice with greater than 90 percent GFP-positive white blood cells (CD45-positive cells) were used for further study. After bone marrow transplantation (week 8), inguinal fat pads of B6 mice were transplanted beneath scalps of GFPBM-B6 mice (B6→GFPBM-B6) (Fig. 1). Transplanted fat was then harvested 12 weeks after grafting.

Immunohistochemistry

Graft samples were fixed (Zinc Fixative; BD Biosciences, San Jose, Calif.), embedded in paraffin, and sectioned at 6 μm. The following primary antibodies were used: rabbit anti-GFP (Novus Biologicals, Littleton, Colo.), guinea pig anti-perilipin (Progen, Heidelberg, Germany), and rat anti-MAC-2 (Cedarlane Laboratories, Burlington, Ontario, Canada). Isotypic antibodies served as negative controls for each marker. Secondary antibodies (anti-rabbit Alexa Fluor 488 or anti-guinea pig Alexa Fluor 568, Life Technologies Corp., Carlsbad, Calif.) were applied as appropriate. Nuclei and vascular endothelial cells were delineated by Hoechst 33342 (Dojindo, Tokyo, Japan) and Alexa Fluor 647–conjugated lectin (Life Technologies Corp.), respectively. Sections were examined via fluorescence (Keyence, Osaka, Japan) or confocal (TCS SP2 system; Leica, Heerbrugg, Switzerland) microscopy.

Whole-Mount Staining

Staining of fresh whole-mount tissue samples was also carried out, as described previously.[9] Briefly, 3-mm sections of adipose tissue were incubated for 30 minutes at 37°C with Alexa Fluor 568–conjugated or 647-conjugated lectin (Life Technologies), targeting vascular endothelial cells, and Hoechst 33342 (Dojindo), directed at nuclei. GFP was detectable without staining. Washed samples were examined via confocal microscopy (Leica TCS SP2 system). Serial images were recorded at 1-μm intervals, producing surface-rendered three-dimensional images.

Stromal Vascular Fraction Isolation

Adipose tissue was minced into 1-mm pieces and digested in phosphate-buffered saline plus 0.075 percent collagenase (Wako Pure Chemical Industries, Ltd., Osaka, Japan) for 30 minutes on a shaker at 37°C. Solutes were then washed, filtered (100-μm and 30-μm mesh), and centrifuged (800 *g* for 5 minutes). Erythrocytes were extracted using lysis buffer (Sigma Aldrich).

Portions of stromal vascular fraction cells were cultured in M199 medium (GIBCO Invitrogen, Carlsbad, Calif.) containing 10% fetal bovine serum, 100 IU of penicillin, 100 mg/ml streptomycin, 5 μg/ml heparin, and 2 ng/ml fibroblast growth factor-1. Contaminants, such as leukocytes and vascular endothelial cells, disappeared after passaging, and only adipose-derived stem/stromal cells were selectively expanded.[17] Adipose-derived stem/stromal cells at second passage were examined under fluorescence microscopy (Keyence) to ascertain GFP-positive cells.

Flow Cytometry Analysis

Stromal vascular fraction cells were identified by surface marker expression via flow cytometry, harvesting stromal vascular fraction cells of three mice for each experiment; analyses were performed in triplicate. Cells were incubated with rat anti-CD34-biotin antibodies (eBioscience, San Diego, Calif.) at 4°C for 30 min. After a wash with phosphate-buffered saline, the following secondary antibody and monoclonal antibody–fluorescein conjugates were applied at 4°C for 30 min: rat anti-streptoavidin-allophycocyanin, rat anti-CD31-fluorescein phycoerythrin (BD Bioscience), and rat anti-CD45-phycoerythrin Cy7 (eBioscience). GFP was detectable without staining. Cells were then analyzed using an LSR II flow cytometry system (BD Biosciences). Adipose-derived stem/stromal cells and vascular endothelial cells were determined as $D45^-/CD34^+/CD31^-$ cells and $CD45^-/CD34^+/CD31^+$ cells, respectively. Inguinal fat pads of normal GFP and B6 mice were also monitored as controls.

There were 4.6 ± 1.9 percent false-positives in B6 mice and 24.0 ± 1.1 percent false negatives in GFP mice (**see Figure, Supplemental Digital Content 1**, which shows a representative flow cytometric analysis of six varied samples. Adipose-derived stem/stromal cells and vascular endothelial cells were determined as $CD45^-/CD34^+/CD31^-$ cells and $CD45^-/CD34^+/CD31^+$ cells, respectively, ***http://links.lww.com/PRS/B296***). Data initially recorded were corrected as follows: *Corrected proportion* [percent] = 100 × (*Naïve data percent – GFP percent of B6 mice*) / (*GFP percent of GFP mice – GFP percent of B6 mice*).

Statistical Analysis

Results are expressed as median (quartile 1 to quartile 3). The Mann-Whitney test was used for

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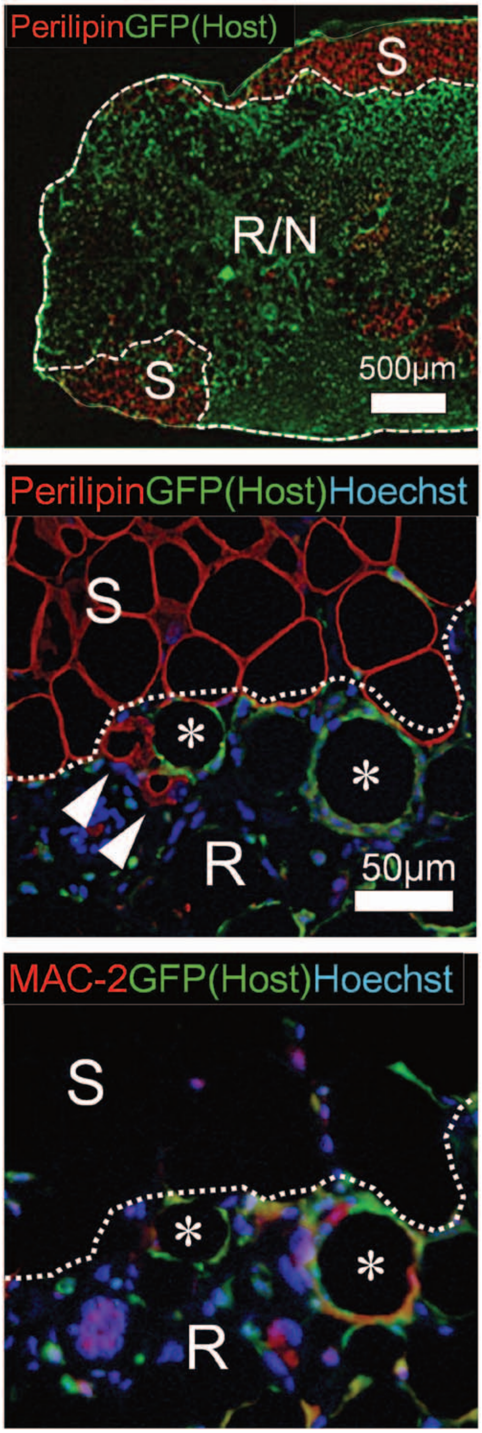

Fig. 2. Adipocyte degeneration/regeneration and host-cell infiltration. Immunostained sequential sections of inguinal fat at 1 week after transplantation are shown. (*Above*) The yellow interrupted line marks the border of surviving (*S*; perilipin-positive) and regenerating/necrotizing (*R/N*; perilipin-negative) tissue. Adipocytes undergo zonal necrosis resulting from ischemia, whereas superficial cells remain viable through plasmatic diffusion from peripheral host tissue. Infiltrating host cells (GFP-positive) predominate in regenerating areas. *Bar* = 500 μm. Sections of B6→GFP model stained for perilipin (adipocyte marker; *center*) and MAC-2 (macrophage marker; *below*) with Hoechst stain and GFP, respectively. Surviving (*S*) and regenerating (*R*) zones of adipocytes are clearly demarcated by perilipin staining (*dotted line*). Host cells infiltrated regenerating areas only and were mostly MAC-2-positive macrophages. The *asterisk* indicates crown-like structure (necrotic adipocytes rimmed by phagocytic macrophages). *Arrowheads* highlight graft-derived (GFP-negative) regenerating adipocytes. *Bars* = 50 μm.

two-group comparisons. Bonferroni correction was used for multiple testing. Statistical significance was otherwise set at $p < 0.05$.

RESULTS

Tissue Regeneration/Remodeling after Fat Grafting

In both exchange models (B6→GFP and GFP→B6), normalized weights of inguinal fat grafts reduced over time since 2 weeks after grafting and was approximately one-half of control at 12 weeks. The graft weight of respective models did not differ statistically at either time point. [**See Figure, Supplemental Digital Content 2**, which shows sequential changes in normalized graft weight of both fat exchange models. Weights of graft samples were normalized, dividing sample weight by body weight. Normalized weight ratios did not differ significantly by graft model (B6→GFP versus GFP→B6). Data are expressed as median (quartile 1 to quartile 3), ***http://links.lww.com/PRS/B297***.]

Grafted adipose tissue underwent total tissue remodeling, as we reported previously.[12,13] Immunohistochemistry of B6→GFP and GFP→B6 models revealed localization of host-derived and graft-derived cells (GFP-positive cells), respectively. With the exception of the most superficial perilipin-positive survival zone (100 to 500 μm from graft surface), all adipocytes were necrotic (perilipin-negative) by 1 week (Fig. 2, *above*). Perilipin is a protein that coats lipid droplets in adipocytes and is detectable only in viable adipocytes.[12] Regenerating and necrotizing zones were then partly replaced by resident and infiltrating stem/progenitor cells. The adipocyte regeneration was complete by 12 weeks.

At 1 week, numerous host cells infiltrated in regenerating/necrotizing perilipin-negative zones but not the viable perilipin-positive survival zone (Fig. 2, *center*). Sequential sections showed that infiltrating host cells were primarily

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MAC-2–positive macrophages, and crown-like structures (necrotic adipocytes rimmed by macrophages) were noted (Fig. 2, *below*). Small (<30 μm), regenerative, perilipin-positive adipocytes were also observed at the peripheries of regenerating zones. Temporal quantification of cell types confirmed that host cells were mainly MAC-2–positive macrophages (Fig. 3), which peaked at 2 weeks (>90 percent) and later declined [62.6 percent (range, 56.3 to 69.9 percent) at 12 weeks; $n = 3$]. At completion of regeneration (12 weeks), scavenging of oil drops was still in progress.

Immunohistologic Assessment of Regenerated Adipocytes

Origins of cells in regenerated adipose tissue were assessed in grafted samples at 12 weeks, with a focus on adipocytes, vessel wall structures, capillaries (vascular endothelial cells), and adipose-derived stem/stromal cells.

In sections of the B6→GFP model, perilipin-negative areas (oil drops) were surrounded by GFP-positive macrophages. However, regenerating perilipin-positive adipocytes barely expressed GFP, suggesting that most of the adipocytes were of graft origin (Fig. 4, *above*). This was corroborated by sections of the GFP→B6 model, in which nearly all perilipin-positive adipocytes expressed GFP (the graft marker here) (Fig. 4, *below*). Furthermore, host-derived adipocytes (GFP-positive/perilipin-negative cells in the B6→GFP model; GFP-negative/perilipin-positive cells in the GFP→B6 model) were exceedingly rare (Fig. 4, *below*), and GFP-positive adipocytes were undetectable in the B6→GFPBM-B6 model. Thus, nearly all regenerated adipocytes were derived from adipocyte progenitors (adipose-derived stem/stromal cells) in grafted tissue, with negligible host-derived contributions.

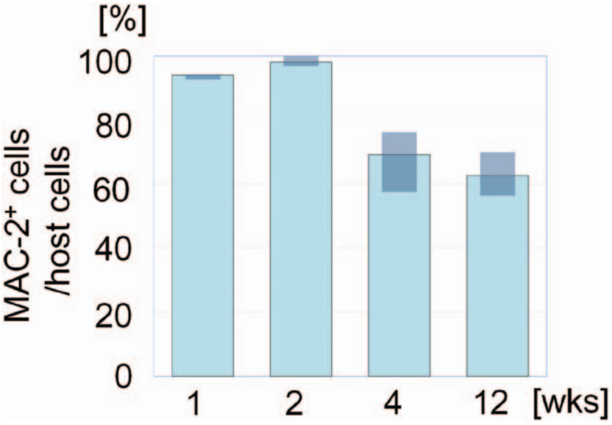

Fig. 3. MAC-2-positive cells quantified relative to all host cells. Nonmacrophage host cells (MAC-2⁻/GFP⁺) rose in number over time. Data are expressed as median (quartile 1 to quartile 3).

Immunohistologic Assessment of Vessel Wall Cells and Capillaries

Large vessels were identified morphologically under phase contrast imaging, and vascular wall smooth muscle cells were evaluated via immunohistochemistry. Host-derived cells accounted for 15.4 percent (range, 11.7 to 17.5 percent) in B6→GFP samplings ($n = 9$) and 36.0 percent (range, 26.1 to 41.8 percent) in GFP→B6 samplings ($n = 9$), indicating a predominance of graft-derived cells (Fig. 5).

Stained whole-mount samples clearly showed that graft-derived and host-derived cells were equally represented in chimeric capillaries of both B6→GFP and GFP→B6 models (Fig. 6, *above* and *center*). This was confirmed by immunostained sections. To further investigate the contribution of host bone marrow–derived cells, whole-mount samples of the B6→GFPBM-B6 model were also stained. Chimeric capillaries, formed through contributing host bone marrow–derived cells (Fig. 6, *below*), were similarly seen.

Flow Cytometric Analysis of Stromal Vascular Fraction

GFP-positive cells were quantified by flow cytometry of stromal vascular fraction cells (i.e., fat constituents other than adipocytes), isolated through collagenase digestion of graft samples. Adipose-derived stem/stromal cells and vascular endothelial cells were identified as $CD45^-/CD34^+/CD31^-$ cells and $CD45^-/CD34^+/CD31^+$ cells, respectively. Representative data of six samples (B6, GFP, B6→GFP, GFP→B6, GFPBM-B6, and B6→GFPBM-B6) are shown in Figure 7 (*above*) and in **Figure, Supplemental Digital Content 1**, ***http://links.lww.com/PRS/B296***. Initial flow cytometry data were corrected as described (see Materials and Methods), and resultant data on cellular origins are shown in Figure 7 (*center*). Our findings suggest that adipose-derived stem/stromal cells were both graft-derived and host-derived, and that host-derived adipose-derived stem/stromal cells were similarly of bone marrow and non–bone marrow derivation. Therefore, surrounding recipient tissue likely supplied some adipose-derived stem/stromal cells during the process of regeneration. Vascular endothelial cells were also composed of graft and host cells to a similar extent, but the host contribution was mostly from bone marrow, with a negligible non–bone marrow component.

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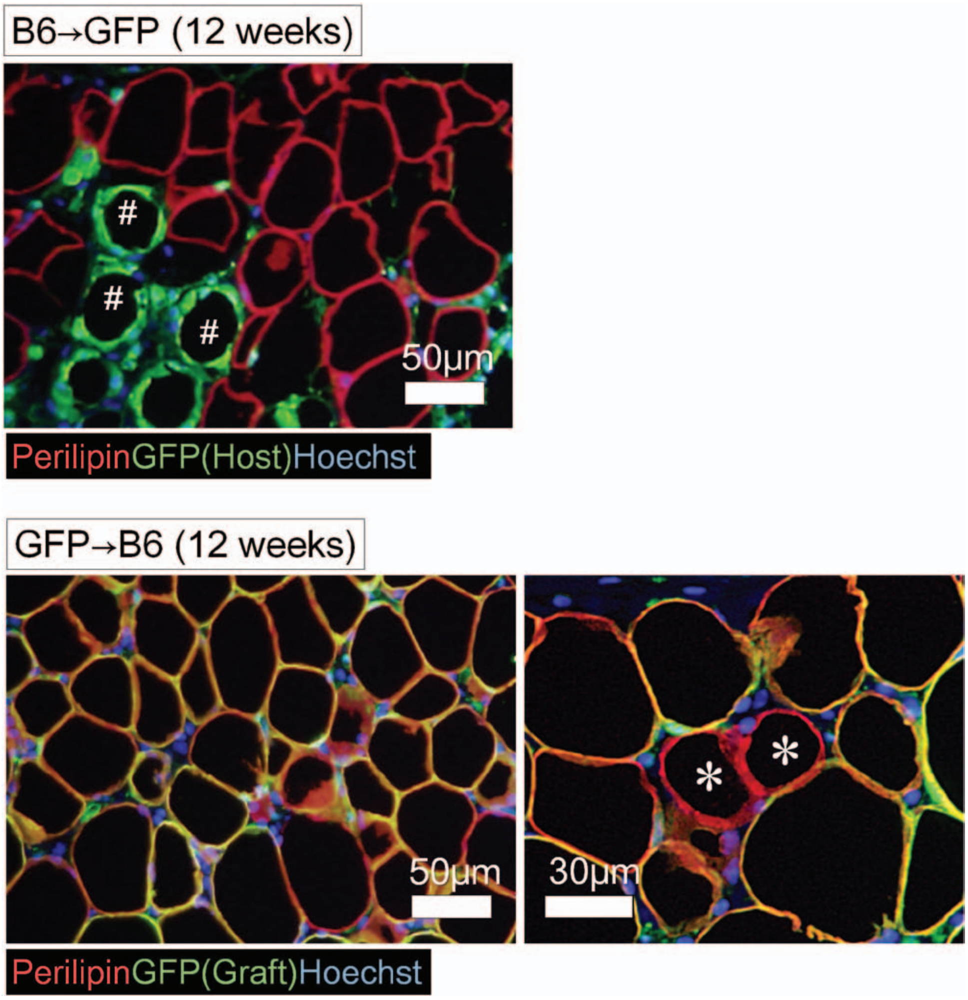

Fig. 4. Origin of regenerated mature adipocytes in exchanged fat. (*Above*) In the B6→GFP model, most mature adipocytes (perilipin-positive) at 12 weeks are GFP-negative (graft-derived). GFP-positive cells surrounding oil drops (#) are chiefly macrophages. *Bars* = 30 μm. (*Below*) In the GFP→B6 model, most mature adipocytes (perilipin-positive) at 12 weeks are GFP-positive (graft-derived) and thus appear yellow (*left*). Host-derived adipocytes (perilipin-positive/GFP-negative) (*) are very rarely detected (*right*). *Bar* = 50 μm (*left*) and 30 μm (*right*).

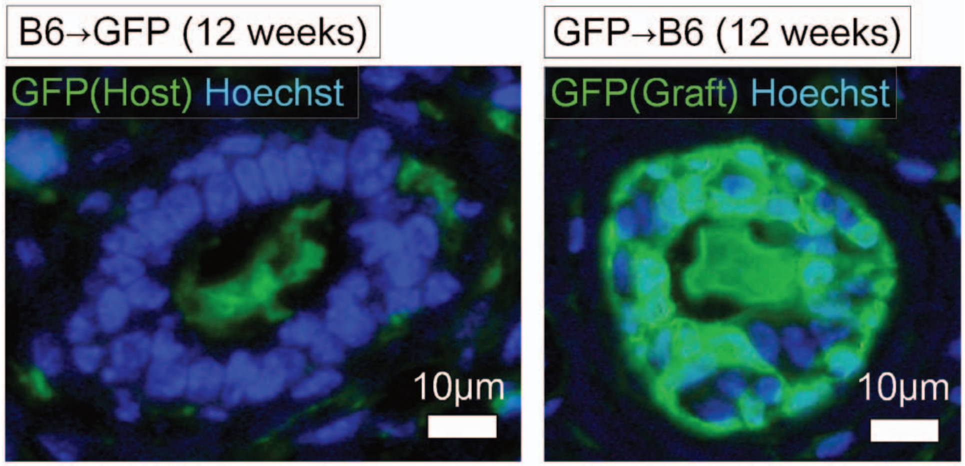

Fig. 5. Origin of vessels and capillaries in exchanged fat tissue. Large vessels chiefly incorporated graft-derived smooth muscle cells in both B6→GFP (*left*) and GFP→B6 (*right*) models. *Bar* = 10 μm.

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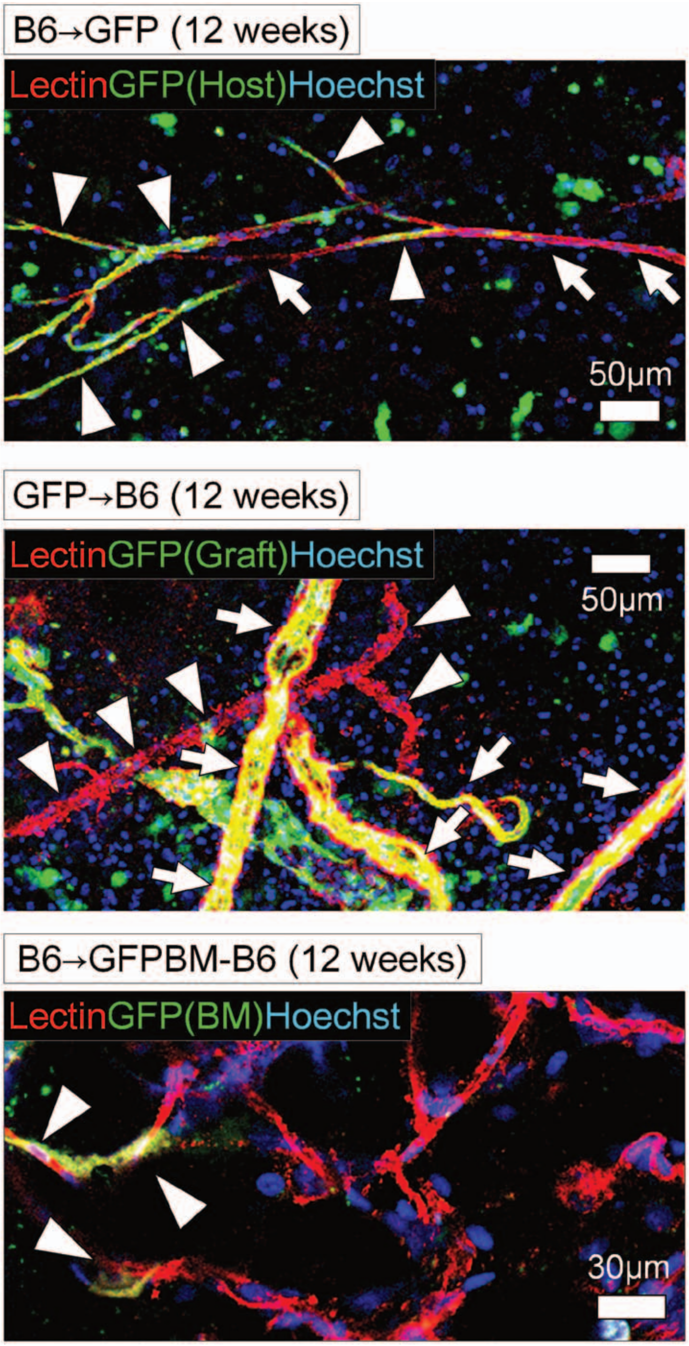

Fig. 6. Origin of vessels and capillaries in exchanged fat tissue. Stained whole-mount samples of B6→GFP (*above*) and GFP→B6 (*center*) models show graft/host chimeric capillaries. *Arrows* indicate graft-derived vascular endothelial (lectin-positive) cells; *arrowheads* highlight host-derived endothelial cells. *Bar* = 50 μm. (*Below*) Stained whole-mount samples of the B6→GFPBM-B6 model showing lectin-positive/GFP-positive host bone marrow–derived vascular endothelial cells. *Bar* = 30 μm.

Stromal vascular fraction cells from B6→GFP and GFP→B6 samples were cultured (Fig. 7, *below, left*). Flow cytometric data of cultured adipose-derived stem/stromal cells were comparable to those of noncultured adipose-derived stem/stromal cells (Fig. 7, *below, right*). Host proportions were 67.7 percent (range, 62.7 to 80.0 percent $n = 3$) and 49.2 percent (range, 33.3 to 67.7 percent; $n = 3$)

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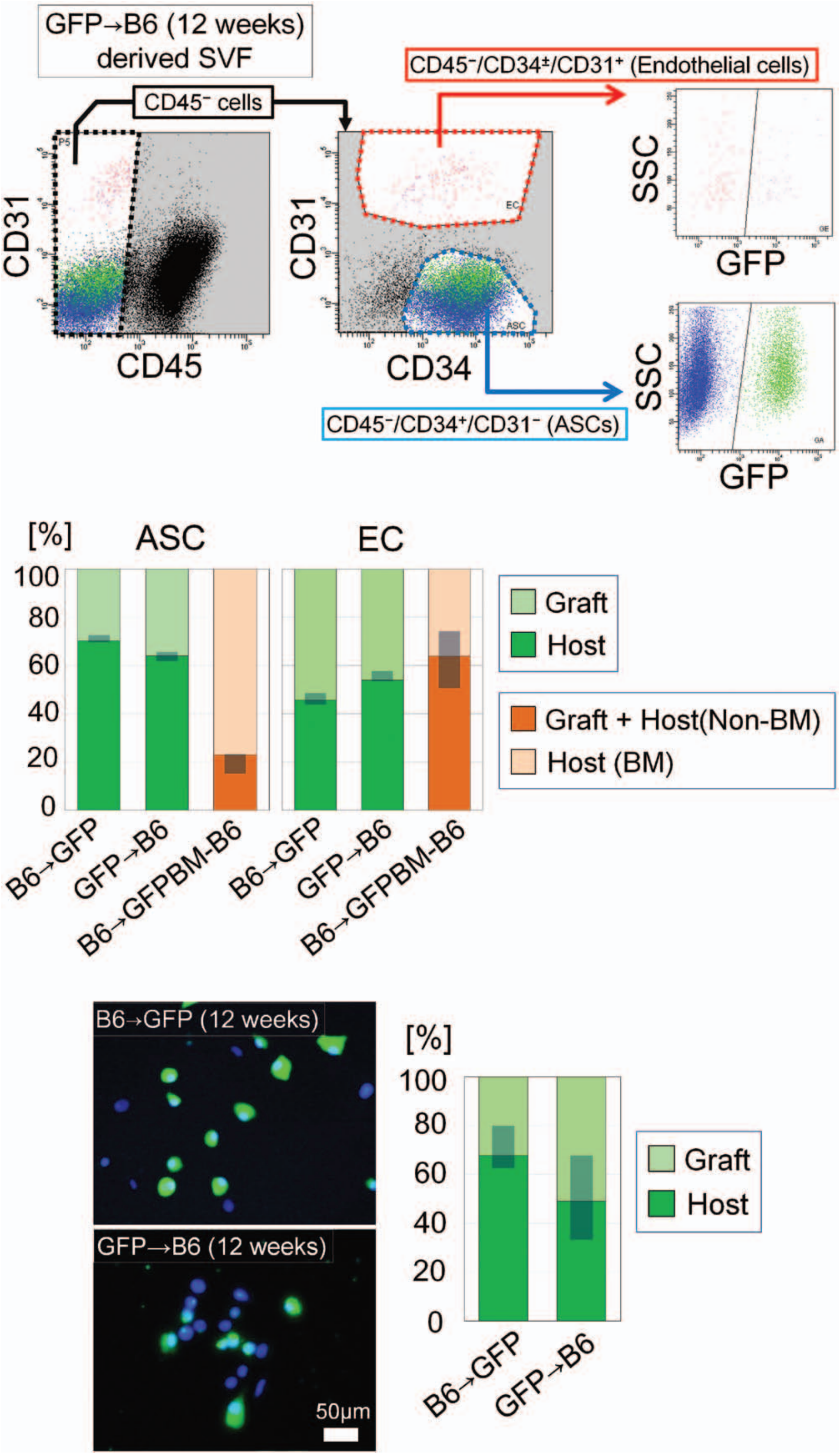

Fig. 7. Analysis of stromal vascular fraction cells isolated from exchanged fat. Freshly isolated or cultured stromal vascular fraction cells from fat samples (three each) of B6→GFP and GFP→B6 models analyzed at 12 weeks. Adipose-derived stem/stromal cells (*ASCs*) and vascular endothelial cells (*ECs*) were determined by flow cytometry ($CD45^-/CD34^+/CD31^-$ cells

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in adipose-derived stem/stromal cells of B6→GFP and GFP→B6 models, respectively.

DISCUSSION

Our previous studies[12,13] suggested that adipose tissue is remodeled during the first 3 months, but the cellular origin of regenerated adipose tissue remained unknown. Using the histological and flow cytometrical data of this study, we summarized the origin of each cellular component in grafted/regenerated fat tissue in Table 1. Except for surviving adipocytes that were superficially located, all other adipocytes are supposed to be regenerated mostly from graft-resident adipose-derived stem/stromal cells. Most of the vascular mural cells are also derived from the graft, whereas nearly half of vascular endothelial cells originate from bone marrow of the host. Adipose-derived stem/stromal cells were a mixture of the graft, the host nonbone marrow, and the host bone marrow. The discrepancy in cellular origin between adipocytes and adipose-derived stem/stromal cells suggested that adipose-derived stem/stromal cells have to reside next to adipocytes to become adipocytes upon adipocyte death, although new adipocytes can be provided from surrounding tissue and bone marrow.

Tissue remodeling in grafted fat was initiated by zonal adipocyte necrosis, triggering adipose-derived stem/stromal cell activation and host cell infiltration by 1 week. Host-cell infiltrates at the early inflammatory phase of repair were almost exclusively macrophages, although by week 12 their percentage had declined to 60 percent, suggesting that other types of cells were involved in later phases of regeneration. Previously, we alluded to the differential roles of inflammatory (M1) macrophages and anti-inflammatory (M2) macrophages in the remodeling of fat.[13,14] M1 macrophages surround and phagocytize necrotic adipocytes (lipid droplets), whereas M2 macrophages appear to take part in scar/capsule formation.

Table 1. Origins of Cellular Components in Grafted Fat

		Host	
	Graft	Non–Bone Marrow	Bone Marrow
Adipocytes	++++	±	–
Vascular mural cells	+++	+	±
Vascular endothelial cells	++	±	++
Adipose-derived stem/stromal cells	++	++	++

Herein, the origins of cellular elements in grafted fat were evaluated at 12 weeks, which reportedly is the time interval required to complete the regeneration of grafts. However, phagocytosis and scar formation are still ongoing beyond this point. Outcomes of our fat exchange graft models clearly indicate the differential contribution of graft-derived and host-derived cells in regenerating/remodeling adipose tissue. New host-derived adipocytes (likely contributed by migrating adipose-derived stem/stromal cells of peripheral host tissue) were rarely detected. Instead, nearly all adipocytes originated from grafts alone. On the other hand (and interestingly), adipose-derived stem/stromal cells found in regenerated fat as future contributors to adipose remodeling were of graft and host origins. Flow cytometric analysis and our experimental bone marrow transplantation in mice suggested that not all host-derived adipose-derived stem/stromal cells originate from bone marrow. A substantial number of stem/progenitor cells likely migrated into grafts from local host tissue as well resided as adipose-derived stem/stromal cells in regenerated fat. Indeed, a previous report has shown that adipose tissue may even release adipose-derived stem/stromal cells into lymphatic flow, and this mobilization of adipose-derived stem/stromal cells may be controlled by CXCR4.[18]

The discrepancy between observed origins of adipocytes and adipose-derived stem/stromal cells is unexplained as yet. Some studies have suggested that bone marrow–derived cells (M2 macrophages) home to adipose tissue and may transform into adipose-derived stem/stromal cells,[19–21] and bone marrow-derived cells are capable of adipocyte differentiation under nonphysiologic experimental conditions, such as a chamber neogenesis model.[22,23] Still, adipocytes

Fig. 7. (*Continued*) and $CD45^-/CD34^+/CD31^+$ cells, respectively). (*Above*) Representative flow cytometric data of stromal vascular fraction cells isolated from GFP→B6 sample at 12 weeks are shown. *SSC*, side scatter. (*Center*) Origins of adipose-derived stem/stromal cells and vascular endothelial cells in samples of B6→GFP, GFP→B6, and B6→GFPBM-B6 models. Flow cytometry confirmed that both adipose-derived stem/stromal cells and vascular endothelial cells were mixtures of graft-derived and host-derived cells. Host-contributed adipose-derived stem/stromal cells were both non–bone marrow– and bone marrow–derived, whereas host-contributed vascular endothelial cells were primarily bone marrow derivatives. Data are expressed as median (quartile 1 to quartile 3) ($n = 3$ at each time point). (*Below, left*) Cultured adipose-derived stem/stromal cells from samples of B6→GFP and GFP→B6 models under fluorescent microscope. (*Below, right*) Graft/host ratio of cultured adipose-derived stem/stromal cells. Data are expressed as median (quartile 1–quatile 3).

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derived from bone marrow are rarely found in mice on normal or high-fat diets.[16,19] Nevertheless, our findings plainly indicate that adipose-derived stem/stromal cells in the grafted tissue are crucial for adipocyte regeneration after fat grafting.

In addition, we found that mural cells (vascular smooth muscle cells) were largely of graft derivation, whereas the origins of vascular endothelial cells were quite different. Both histologic and flow cytometric analyses revealed a mix of graft and host origins for these cells. Unlike adipose-derived stem/stromal cells, our experimental transplantation of mouse bone marrow suggested that nearly all host-derived vascular endothelial cells originate from bone marrow. This means that a substantial number of endothelial progenitor cells are mobilized from bone marrow, resulting in a capillary network chimera. Similar graft/host chimeric capillaries have been observed after kidney transplantation.[24] Hence, capillary remodeling in the aftermath of fat grafting technically is a culmination of vasculogenesis, rather than angiogenesis.

CONCLUSIONS

The clinical implications of this study are considerable in terms of homeostasis/remodeling in adipose tissue and repair/regeneration of grafted fat. Next-generation adipocytes in fat grafts are derived from adipose-derived stem/stromal cells in the graft, but new adipose-derived stem/stromal cells for future remodeling are also provided by bone marrow and other local (adipose) tissue elements. Adipose tissue may also serve as a reservoir of adipose-derived stem/stromal cells from circulation. Although graft-derived vascular structures are involved in revascularization after fat grafting, endothelial progenitor cells mobilized from bone marrow are major contributors as well. We believe that understanding the mechanisms of fat grafting would strategically improve current grafting procedures. Our findings also propose future studies that explore novel methods to manipulate activation, migration, or homing of graft-derived and bone marrow–derived stem/progenitor cells.

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ACKNOWLEDGMENTS

This study was supported by the Japanese Ministry of Education, Culture, Sports, Science, and Technology (Grant-in-Aid for Scientific Research B3-24390398). The authors greatly appreciate the substantial contributions to this study by Drs. Kazuhide Mineda and Takanobu Mashiko. They also thank Ayako Sato for technical assistance.

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TRANSPLANTATION

Daily Topical Tacrolimus Therapy Prevents Skin Rejection in a Rodent Hind Limb Allograft Model

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Background: Skin is the most immunogenic component of a composite tissue allograft. Topical immunotherapy is an attractive therapeutic modality with which to provide local immunosuppression, with minimal systemic toxicity. The present study was performed to investigate the potential of topical tacrolimus to prolong survival of the skin component of a composite tissue allograft.
Methods: Wistar Furth–to-Lewis rat orthotopic hind limb transplants were performed. Group I consisted of rats treated with topical tacrolimus; group II, antilymphocyte serum plus 21 days cyclosporine; and group III, antilymphocyte serum plus 21 days of cyclosporine plus topical tacrolimus. In group IV, tacrolimus levels in blood, skin, and muscle were measured in an autograft control group.
Results: All animals in group I ($n = 8$) developed grade III clinical rejection by postoperative day 9. In group II ($n = 9$), the median onset of grade III rejection was postoperative day 40 (range, postoperative days 34 to 44). In group III ($n = 6$), two animals developed focal grade III rejection on postoperative days 35 and 56. The remaining four animals reached the 100-day endpoint without grade III rejection. In group IV, tacrolimus levels were low or undetectable in blood, whereas skin levels were 100-fold higher than underlying muscle.
Conclusions: Topical tacrolimus therapy has the potential to prevent skin rejection in a composite tissue allograft. Preoperative depletion of T cells with antilymphocyte serum, along with a short course of systemic immunosuppression, prevents acute rejection, whereas topical tacrolimus inhibits immune cell function in the skin. Concentrations of tacrolimus are substantially higher in skin compared with underlying muscle and peripheral blood. Topical immunotherapy could reduce the morbidity associated with systemic immunosuppression in clinical composite tissue allografts. (*Plast. Reconstr. Surg.* 123 (Suppl.): 17S, 2009.)

Skin is the most immunogenic component of a composite tissue allograft. Clinically, this has manifested as multiple acute skin rejection episodes in most of the human hand and facial allografts performed to date.[1] Intravenous steroids and increased systemic immunosuppression have been used to mitigate these rejection episodes. These drugs cause direct organ toxicity, such as nephrotoxicity and neurotoxicity, and are associated with undesirable sequelae such as metabolic dysfunction, opportunistic infection, and malignancy. Topical immunotherapy is an attractive and practical therapeutic option with which to provide local immunosuppression, with minimal systemic toxicity.

Tacrolimus (FK506) is a macrolide lactone derived from the fungus *Streptomyces tsukubaensis*. As a calcineurin inhibitor, its major effect is on T

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Received for publication November 27, 2007; accepted June 13, 2008.
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DOI: 10.1097/PRS.0b013e318191bcbd

Disclosure: *None of the authors has any commercial associations or financial disclosures that might pose or create a conflict of interest with information presented in this article.*

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