

Adipose Stromal Cells and Platelet-Rich Plasma Therapies Synergistically Increase Revascularization during Wound Healing

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Background: The authors examined the efficacy of adipose stem cells, when supplied either alone or in platelet-rich fibrin gels, to improve wound healing. **Methods:** A porcine full-thickness wound model was used to compare six topical treatments: platelet-poor plasma; platelet-rich plasma; autologous adipose stem cells plus platelet-poor plasma; autologous adipose stem cells plus platelet-rich plasma; allogeneic adipose stem cells containing green fluorescent protein plus platelet-poor plasma; and saline (control). One week after isolation, adipose stem cells were applied to full-thickness wounds on the paraspinal and thoracic regions of three pigs (44 wounds per pig; each treatment was applied to eight separate wounds). Each wound was monitored over 21 days for closure, cosmesis, and histopathology.

Results: There was no significant difference in the reepithelialization rate, but treatments containing adipose stem cells demonstrated increased microvessel densities (31.75 ± 5.73 vessels/cm² versus 7.93 ± 3.61 vessels/cm²) compared with groups without adipose stem cells. Wound cosmesis was improved in the adipose stem cell plus platelet-rich plasma group compared with other treatment groups ($p < 0.05$). Vascular endothelial growth factor levels detected in matrices containing adipose stem cells were approximately 7-fold higher compared with platelet-rich plasma or platelet-poor plasma ($p < 0.05$). Localization of transgenic green fluorescent protein plus adipose stem cells indicated incorporation near neovasculation.

Conclusions: In normal healing wounds, adipose stem cells appear to enhance the healing process only when provided in a fibrin gel vehicle containing a number of complementary wound-healing trophic factors. Perivascular adipose stem cell localization suggests a function in enhancing blood supply through providing physical and paracrine support to newly forming vessels. (*Plast. Reconstr. Surg.* 123 (Suppl.): 56S, 2009.)

Despite modern advances in wound closure techniques and devices, and wound maintenance, there is still a critical need for new methods of enhancing the healing process to achieve optimal outcomes. One of the promising yet clinically challenging areas of recent therapeutic

development involves topical application of growth factors to enhance the normal healing process.¹ Many of these new therapies have involved the provision of individual trophic factors with defined biological activities. They have a restricting growth factor release that is dependent on matrix loading, thus potentially limiting their overall ability to affect healing. Potentially more desirable for optimal wound healing would be therapies that augment the normal healing re-

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sponse by continuously supplying a mixture of factors mimicking the natural milieu. Platelets contain large stores of cytokines and growth factors that are normally released during clot formation at wound sites. Concentrates of platelets from plasma (platelet-rich plasma) are used for a wide variety of surgical applications, particularly in cosmetic and maxillofacial procedures.²

Therapeutic effects of platelet-rich plasma are believed to occur through the provision of concentrated levels of platelet-derived growth factors, such as platelet-derived growth factor (PDGF)-BB, transforming growth factor (TGF)- β 1, vascular endothelial growth factor (VEGF), and epidermal growth factor (EGF).² In addition, the fibrin matrix that is generated on activation may potentially aid in tissue repair by providing a scaffold for tissue ingrowth.

Adipose-derived stem (stromal) cells are an abundant population of pluripotent cells found in the stroma of adipose tissues that have been shown to differentiate *in vitro* into various cell lineages, including osteogenic, myogenic, neurogenic, and hemopoietic.^{3,4} We have also demonstrated that adipose stem cells are a robust source of bioactive growth factors that contribute to recovery from ischemic damage through preservation of skeletal muscle and restoration of blood flow.³ The potential for therapeutic translation using adipose stem cells is high given the ease with which they can be harvested in high yield using simple, minimally invasive lipoaspiration procedures and re-applied as an autologous therapy with minimal need for manipulation or expansion.⁵

We hypothesized that an improved tissue defect repair or augmentation of underlying support tissues would be gained by mixing platelet-rich plasma (or other matrices) with autologous adipose stem cells. This study thus examined the beneficial effects of treating full-thickness wounds with adipose stem cells immobilized in fibrin matrices derived from platelet concentrates.

MATERIALS AND METHODS

Autologous Adipose Tissue Harvest

Female Yorkshire pigs ($n = 3$) weighing 40 to 45 kg were sedated with a mixture of ketamine (10 mg/kg) and midazolam (1 mg/kg) followed by endotracheal intubation and maintained under a surgical plane of anesthesia with 1 to 2% isoflurane and a 50:50 mixture of nitrous oxide and oxygen (3 to 5 liters/minute) for the adipose harvest. The dorsal hair was removed with hair clip-

pers and the skin was swabbed clean with chlorhexidine and 70% isopropyl alcohol solution.

One 10-cm incision was made into the dorsal hump of each pig to a depth of 3 to 4 cm followed by adipose tissue excision (15.7 ± 4.5 g) with a no. 10 blade scalpel. The fat was transferred to sterile 50-ml conical tubes (Fisher Scientific, Pittsburgh, Pa.) and transported promptly at room temperature for processing. Wound hemostasis was maintained with electrocautery followed by reapproximation of the deep dermal tissue with interrupted 3-0 Vicryl (Ethicon, Inc., Somerville, N.J.) and a running 4-0 Vicryl subcutaneous closure. Each incision was covered with an occlusive dressing (Tegaderm; 3M Health Care, St. Paul, Minn.).

Preparation of Adipose Stromal Cells

The adipose tissue was minced into small pieces (approximately 1 cm³) and digested in 50 ml of buffer containing 2 mg/ml type I collagenase (Worthington, Lakewood, N.J.) with intermittent shaking in a water bath at 37°C for 180 minutes. Digestion was neutralized by the addition of culture medium (Dulbecco's Modified Eagle Medium, 10% fetal bovine serum, penicillin/streptomycin, amphotericin-B, and gentamicin). This cell suspension was centrifuged (300 *g* for 7 minutes at 25°C) followed by removal of the supernatant and resuspension of the cell pellet in fresh Dulbecco's Modified Eagle Medium culture medium with 10% fetal bovine serum and penicillin/streptomycin, amphotericin-B, and gentamicin. This resuspension was filtered with a 100- μ m nylon cell strainer (GIBCO, Carlsbad, Calif.) followed by another centrifugation (300 *g* for 7 minutes at 25°C) and supernatant removal with the resultant cell pellet used for culture. Before the cells were placed in the culture flasks, they were counted with a hemocytometer and viability was determined using trypan blue exclusion. On reaching 90 percent confluence, the isolated cells were trypsinized, counted, and subcultured for two passages using a 1:5 plating ratio. At passage 2 (7 days), cells were trypsinized, counted, and prepared for the appropriate treatment applications.

Preparation of Wound Treatment Combinations

On the day of surgery, platelets were separated from 55 ml of autologous peripheral blood taken from the femoral artery of each pig. Briefly, the blood was mixed with anticoagulant citrate dextrose solution A (Citra, Braintree, Mass.) and placed into a Gravitational Platelet Separation de-

vice (GPS II; Biomet Biologics, Inc., Warsaw, Ind.). The blood was separated by means of a single, 12-minute centrifuge spin, reducing the total volume to approximately 6 ml of platelet-rich plasma and 30 ml of platelet-poor plasma per 55 ml of separated whole blood. Three milliliters of either platelet-rich plasma (platelet count, $998 \pm 39.8 \times 10^3/\mu\text{l}$) or platelet-poor plasma were admixed with autologous adipose stem cells (cell count, $18.3 \pm 1.87 \times 10^5$) to yield their respective treatment suspensions (platelet-poor plasma only, platelet-rich plasma only, adipose stem cells plus platelet-poor plasma, adipose stem cells plus platelet-rich plasma, and green fluorescent protein–adipose stem cells plus platelet-poor plasma) and each was placed into a FibriJet surgical sealant applicator (Micromedics, Germany). A 20-gauge FibriJet dual cannula applicator tip was placed on all applicators. According to the GPS II instructions, a solution of 5000 IU topical thrombin (Jones Pharma, Inc., Bristol, Va.) and 5 ml of 10% calcium chloride was placed in the complementary compartment of the applicators using a 10:1 volume ratio of platelet-rich plasma, platelet-poor plasma, and adipose stem cell treatment combinations to the thrombin/calcium chloride solution.

Wound Model and Treatment Procedure

Seven days after adipose tissue harvest, the same three female Yorkshire pigs were weighed, sedated, intubated, prepared, and anesthetized as above. After thoracic and dorsal hair was removed with hair clippers and the skin cleaned with chlorhexidine and 70% isopropyl alcohol solution, a template was used to define the wound sites, which were organized in four rows on the lateral paraspinal and thoracic areas (Fig. 1, *above*). Before wounding, each wound site was demarcated with a sterile marker (Fig. 1, *center*) and the rows and columns were labeled with tattoo dye. A no. 15 blade was used to excise a 1.5-cm² wound, taking care not to injure the underlying musculature. The full thickness of skin and underlying subcutaneous layers was removed and covered with moist gauze to avoid desiccation. The wounds had an average depth of approximately 7.9 ± 1.2 mm. Each pig had 22 wounds created on the left and right sides, thus creating a total of 44 wounds per pig and a grand total of 132 wounds in the study (Fig. 1, *below*).

Each wound was treated randomly with one of the following treatments: (1) adipose stem cells and platelet-poor plasma; (2) adipose stem cells

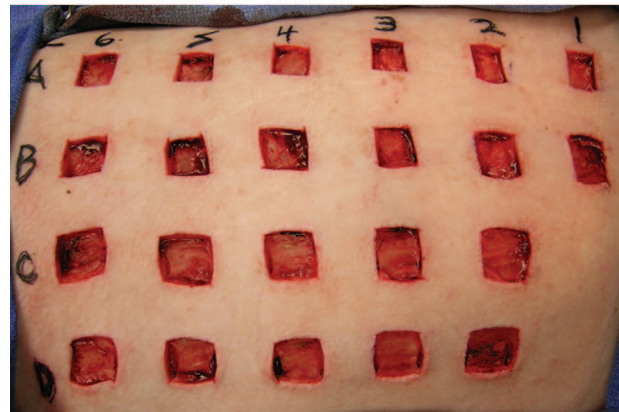
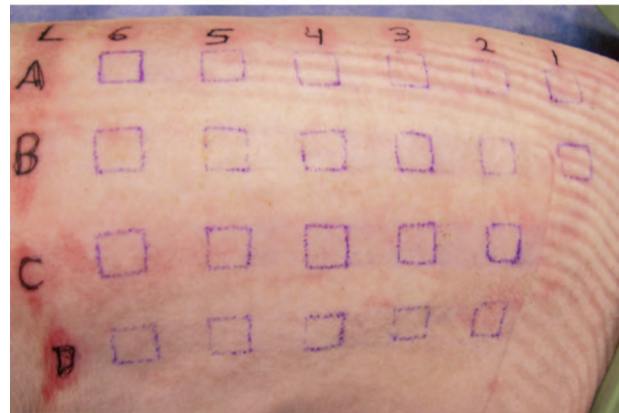
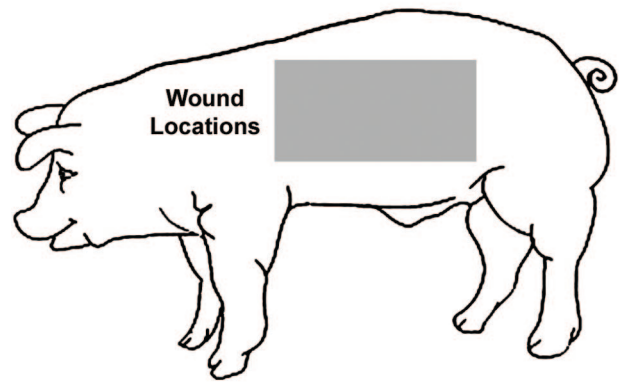


Fig. 1. Wound placement and wounding patterns used. (*Above*) Schematic shows the relative anatomical location of the bilaterally wounded regions. (*Center*) The layout of the 1.5 × 1.5-cm full-thickness wound sites ($n = 22$) that were marked on the paraspinal and lateral thoracic skin regions of both sides of the pig. (*Below*) Appearance of a pig obtained immediately after creation of the wounds. The average thickness of each wound was 7.8 ± 0.4 mm.

and platelet-rich plasma; (3) platelet-poor plasma; (4) platelet-rich plasma; (5) green fluorescent protein and adipose stem cells in platelet-poor plasma; and (6) saline. Each treatment was applied to eight separate wounds. The wound was then quickly covered with an occlusive dressing (Tegaderm) to avoid runoff and treatment loss.

Each wound's volume was completely filled with treatment (approximately 0.8 ml). The thorax and abdomen were then covered with a snug-fitting, nonadherent tube gauze dressing followed by a modified cotton vest (Four Flags Over Aspen, Jamesville, Minn.) to further protect the wounds. The subjects were then awakened and allowed to recover appropriately. Of note, all animals received cephalexin (50 mg/kg) 30 minutes before wounding and a fentanyl patch (25 µg) for 72 hours. Antibiotic prophylactic coverage was continued for the duration of the subject's postoperative survival (21 days). Animal care was performed according to *Guidelines for the Care and Use of Laboratory Animals* published by the National Institutes of Health, and the protocol was approved by the institutional animal care committee.

Wound Assessments

The wounds were examined on postwounding day 3, 7, 14, and 21. The subjects were sedated with a subcutaneous injection of ketamine (10 mg/kg) and midazolam (1 mg/kg) followed by cleansing of the normal skin with saline only, with careful attention paid to not débride within the wound bed. Qualitative wound assessments were recorded and individual digital photographs were taken. Wounds were measured by ruler to the nearest millimeter, and open surface area was calculated and expressed as a percentage of the original wound area on the day of wounding. Punch biopsies were performed on postwounding days 3, 7, 14, and 21. These were 5 to 6 mm in width, mediolaterally oriented, and included approximately 3 to 4 mm of nonwounded skin on both sides of the wound. All biopsy samples were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned, and then examined histologically. At that time, all wounds, both those that had undergone biopsy and those that had not undergone biopsy, were redressed with adhesive and Tegaderm dressings. Once a wound had undergone biopsy, it was not included in the additional assessments. The pigs were then allowed to recover from anesthesia.

Macroscopic Evaluation of Wound Healing

On the final wound assessment day (day 21), a modified subjective wound evaluation score was performed to assess the quality of the scar. Two independent experienced observers scored the wounds on a five-point scale for wound color (pink to purple/red), smoothness, and wound suppleness while blinded to the treatment pattern. The

overall score ranged from 1 (like normal skin) to 5 (excessive scarring).⁶

Histologic and Immunohistochemical Analysis of Wound Healing

Hematoxylin and eosin stains were used to visualize cell infiltration and dermal tissue architecture by a board-certified veterinary pathologist blinded to treatment modalities. For immunohistochemistry, the 5-µm sections taken from the day-14 punch biopsy specimens were stained with anti- α -smooth muscle actin to determine vessel density. The sections were deparaffinized and stained according to a standard three-step immunohistochemical procedure.⁷ The primary antibody used was a monoclonal anti- α -smooth muscle actin clone (1A4; Sigma, St. Louis, Mo.). After washing with phosphate-buffered saline containing 0.05% Tween 20, samples were incubated with conjugated anti-mouse immunoglobulin G antibodies (Vector Elite Kit PK-6102; Vector Laboratories, Burlingame, Calif.) followed by development with diaminobenzidine as substrate and counterstaining with hematoxylin A. As negative controls, adjacent sections were stained with non-immune immunoglobulin G from the same species using the same dilution as the primary antibody. No specific signal was noticed in the negative controls. Sections from normal porcine skin served as positive controls.

Image analysis of sections stained for α -smooth muscle actin analysis was performed using white light and a low-magnification objective (4 \times). Images were recorded with a color camera (QImaging RETIGA EXi FAST; Burnaby, British Columbia) attached to a Nikon Eclipse TE2000-5 microscope. Vessel density was determined by counting for vascular structures stained with α -smooth muscle actin as outlined in the immunohistochemistry procedures above. Tissue area and number of positively stained lumen-containing vessels were measured using three random fields per slide viewed at high-magnification (20 \times). The images were viewed with ImageJ software, and blood vessels in each high-powered field were marked and counted.

Tracking Green Fluorescence Protein-Labeled Adipose Stem Cells in Wound Treatment Application

Green fluorescence protein-labeled porcine adipose tissue was obtained from transgenic Yorkshire pigs expressing green fluorescence protein under control of the ROSA26 promoter, from the

National Swine Resource and Research Center (University of Missouri), followed by adipose stem cell isolation and nonautologous treatment preparation as described above. The green fluorescence protein–adipose stem cell fluorescence was verified after adipose stem cell isolation and before treatment application. A total of 12 wounds were reserved for the allogeneic transgenic green fluorescence protein–adipose stem cell treatments. Punch biopsy specimens were taken at the same biopsy time points and underwent immunohistochemical analysis. The 5- μ m sections were deparaffinized and blocked with A/B block (Vector) at room temperature for 15 minutes. The primary antibody was a mouse anti-human monoclonal green fluorescent protein antibody (1:800 dilution; Clontech, Mountain View, Calif.). After washing with phosphate-buffered saline containing 0.05% Tween 20, samples were incubated with streptavidin (LSAB+ Kit; Dako, Carpinteria, Calif.), rinsed, and incubated with diaminobenzidine (Dako). Evident positive immunoreactivity was seen in green fluorescence protein–transgenic porcine skeletal muscle, with no staining in the nonimmune control.

In Vitro Enzyme-Linked Immunosorbent Assay Analysis of Growth Factors

Growth factor levels for human PDGF-BB, porcine TGF- β 1, and human VEGF concentrations were measured in vitro for all treatment combinations. The treatments were prepared as above and sprayed into culture flasks. Basal Dulbecco's Modified Eagle Medium devoid of growth factors was added (10 ml), and the supernatant was collected after 72 hours. The growth factor concentrations from all treatment supernatants were determined using the enzyme-linked immunosorbent assay method, tested according to the manufacturer's instructions (R&D Systems, Minneapolis, Minn.), and measured using a microplate reader (Fusion; PerkinElmer, Shelton, Conn.).

Statistical Analysis

A randomized complete block design was used to account for potential dependence of multiple treatments for each pig. For this design, each pig was identified as a block (three blocks total) and each block was divided into 40 wound sites that received five different treatments assigned randomly to the 40 sites. All five treatments were observed within each block. All results, with the exception of the macroscopic analysis, were analyzed for significant differences with an unpaired

t test (two-sided). A value of $p < 0.05$ was considered statistically significant. The macroscopic analysis was analyzed using a Kruskal-Wallis test followed by the Mann-Whitney *U* test, with a value of $p < 0.05$ considered statistically significant.

RESULTS

Treatment with Adipose Stem Cells Improves the Cosmetic Appearance of Healed Wounds but Not Wound Closure Rate in Healthy Juvenile Pigs

All wounds were evaluated for healing properties throughout the 3-week period by visual inspection, palpation, and analysis of biopsy specimens. The epithelial overgrowth in all wounds was similar, and closure was nearly complete at 3 weeks (Fig. 2). The wound colors changed from red/purple to light pink by 21 days and became comparable in color and texture to the surrounding skin in all groups (Fig. 3). All treatment groups revealed a trend toward improved cosmesis; however, only the adipose stem cell plus platelet-rich plasma group displayed a significant improvement in the qualitative scoring scale when compared with the adipose stem cell plus platelet-poor plasma, platelet-rich plasma, and control treatments (Fig. 4) ($p < 0.05$). The adipose stem cell-treated wounds depicted a normal healing process, proceeding from acute injury with mild inflammation at day 3 to early granulation tissue and partial epithelial covering by day 8 to nearly complete to complete epithelial covering of the

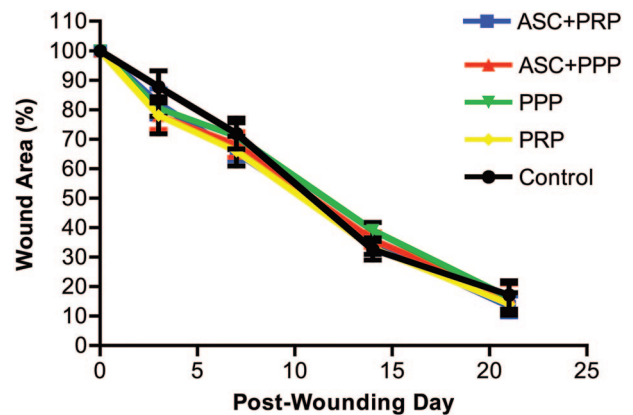


Fig. 2. Comparison of wound-healing rates between treatment groups. All wounds were measured weekly using digital calipers. The wound contraction rate is plotted as the percentage reduction of original wound area over time. The measurements were corrected for the local regional growth of the animal. There was no significant difference in contraction rates among all treatment groups ($p > 0.05$).

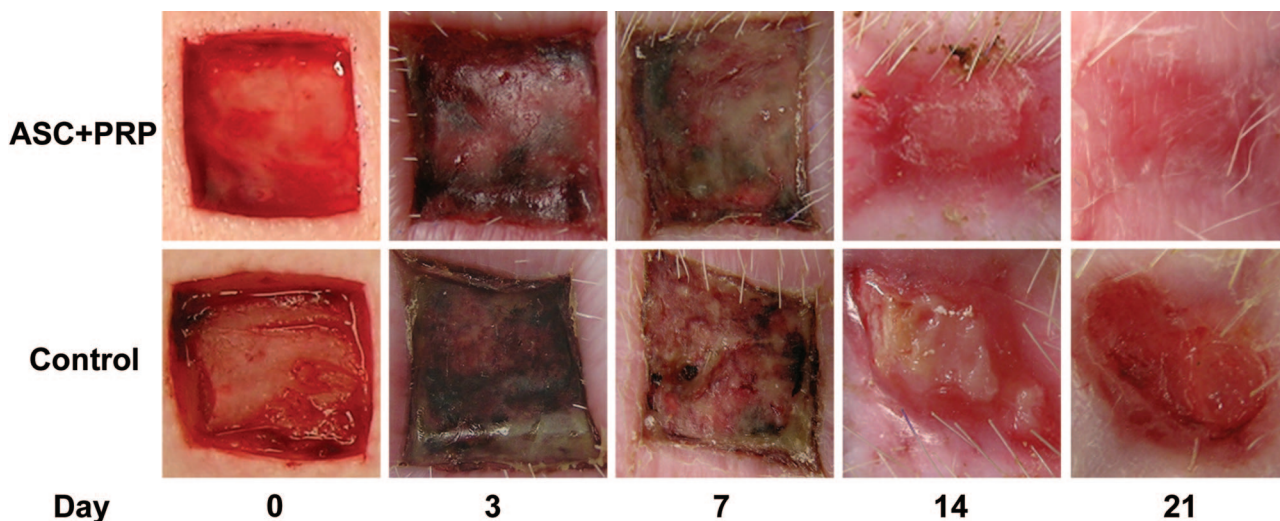


Fig. 3. Appearance of wounds treated with adipose stem cells plus platelet-rich plasma and with control during the 21-day study. The adipose stem cell plus platelet-rich plasma–treated wounds had uniform color and elasticity comparable to surrounding skin. The control group had decreased elasticity and unhealed wound parameters.

defects by days 16 to 21. Granulation tissue matured to more dense collagenous connective tissue

by day 21. No hyperplastic, keloid, or premalignant skin changes were observed.

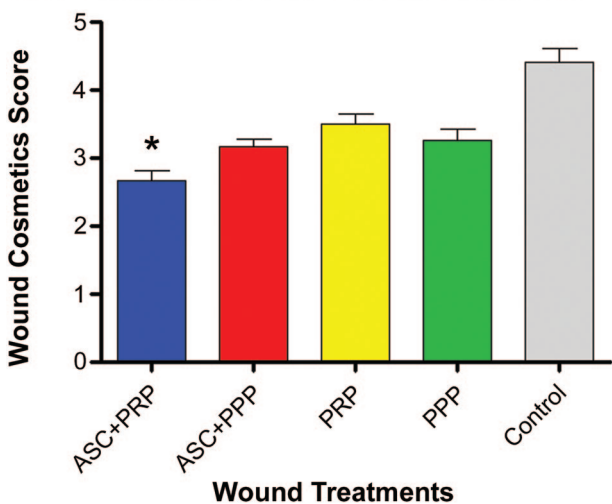


Fig. 4. Macroscopic wound analysis at 3 weeks after wounding comprised a subjective wound evaluation score (scale of 1 to 5) by two independent experienced observers scoring wound color (pink to purplish-red), smoothness, and wound suppleness or stiffness. (Above) A representative image of wounds from a single pig showing the range of wound characteristics which were assessed macroscopically. (Below) Average (\pm SEM) wound cosmetic score based on bilateral observations from three pigs ($n = 6$ for each treatment). The wounds treated with adipose stem cells plus platelet-rich plasma scored significantly better for cosmetic appearance than those treated with saline control ($*p < 0.05$).

Vessel Density Increases following Introduction of Adipose Stem Cells into Wounds

The microvascular density within the healed wounds was examined in biopsy specimens taken 21 days after wounding and treatment. The number of α -smooth muscle actin–positive lumen-containing vascular structures in the group receiving adipose stem cells was significantly greater compared with treatments not containing adipose stem cells (Fig. 5, above). Significantly more vessels were apparent in the adipose stem cell plus platelet-rich plasma–treated wounds than all other groups, including the adipose stem cell plus platelet-poor plasma, suggesting an augmented effect of adipose stem cells plus platelet-rich plasma ($p < 0.05$) (Fig. 5, below).

Enhanced Vascularity in the Adipose Stem Cell plus Platelet-Rich Plasma Treatment Groups Correlates with Higher VEGF Levels

To determine whether a correlation existed between particular growth factor levels and wound-healing responses, we examined in vitro levels of several growth factors in each treatment group. The factors assayed, TGF- β 1, PDGF-BB, and VEGF, were selected based on their previously demonstrated importance in improving wound healing^{8–10} and the availability of enzyme-linked immunosorbent assay reagents that detect the porcine proteins. The growth factor concentrations of

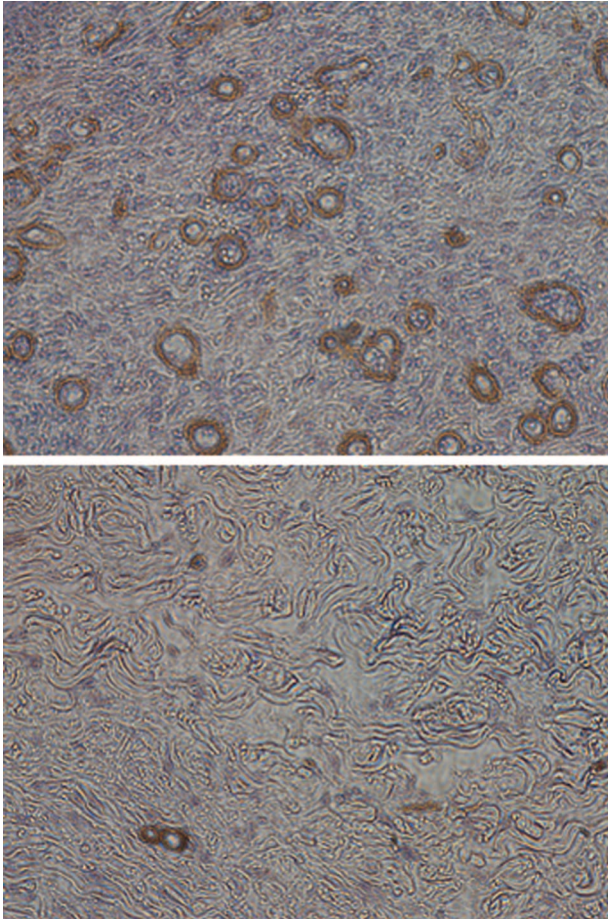


Fig. 5. Assessment of microvessel densities in the repaired wounds receiving the various treatments. (Above) Immunohistochemical visualization of α -smooth muscle actin-containing microvessels in 3-week postwounding biopsy specimens. (Below) Quantification of microvascular densities in the wounds treated with the indicated agents. Digitized high-power (200 \times) photomicrographic images were used to quantitate vascular structures. Quantification is expressed as the number of smooth muscle actin-positive vessels per high-power field ($p < 0.05$ compared with platelet-rich plasma, platelet-poor plasma, and control groups; $p < 0.05$ compared with all other groups).

the different treatment groups after 72-hour culture are listed in Table 1.

Very low to undetectable levels of TGF- β 1 (290 ± 30 pg/ml), PDGF-BB (0 pg/ml), and VEGF (0 mg/ml) were released from platelet-poor plasma into the culture medium. In contrast, abundant amounts of PDGF-BB (860 ± 100 pg/ml) and TGF- β 1 ($12,000 \pm 940$ pg/ml) were released from the activated platelet-rich plasma matrix. A slight but statistically insignificant enhancement in TGF- β 1 was observed in activated platelet-rich plasma matrix containing adipose stem cells ($15,980 \pm 580$ pg/ml). The adipose stem cells did not secrete detectable levels of PDGF-BB when cultured alone or within platelet-poor plasma. Similarly, PDGF-BB levels released from platelet-rich plasma were essentially unchanged by the addition of adipose stem cells (1170 ± 100 pg/ml). Conversely, adipose stem cells secreted significantly ($p < 0.05$) higher levels of VEGF (1100 ± 190 pg/ml) into the medium than were released by either platelet-rich plasma (140 ± 10 pg/ml) or platelet-poor plasma (0 pg/ml). The levels of VEGF were not influenced by entrapment of adipose stem cells in the fibrin matrix only (platelet-poor plasma); interestingly, though, a 7-fold increase in VEGF released to the medium was observed when adipose stem cells were imbedded in platelet-rich plasma (1830 ± 70 pg/ml). Attempts to liberate adipose stem cells for counting were not successful; therefore, it is not known whether the observed enhancement was attributable to increased production of VEGF on a per-cell basis or an increase in cell numbers. Overall, the data for trophic factor levels released by the different treatments, as measured in vitro, suggest that enhanced vascularization of repaired wounds treated with adipose stem cells plus platelet-rich plasma may be at least partially explained by locally enhanced levels of VEGF.

Transgenic Green Fluorescent Protein–Adipose Stem Cells Localize to the Vasculature in Newly Formed Dermal Tissue

Histopathologic analysis of thin sections taken from wounds treated with green fluorescent pro-

Table 1. Growth Factor Concentrations in Wound Treatment Samples at 72 Hours as Determined by Enzyme-Linked Immunosorbent Assays

Growth Factor	Wound Treatments				
	ASC + PRP	ASC + PPP	ASC	PRP	PPP
VEGF (pg/ml)	$1830 \pm 70^*$	974 ± 12	$1100 \pm 190^*$	140 ± 10	0
TGF- β 1 (pg/ml)	$15,980 \pm 580$	1630 ± 56	1840 ± 190	$12,000 \pm 940$	290 ± 30
PDGF BB (pg/ml)	1170 ± 100	1 ± 0.30	0	860 ± 100	2 ± 0.30

PRP, platelet-rich plasma; PPP, platelet-poor plasma; ASC, adipose stem cells.

* $p < 0.05$.

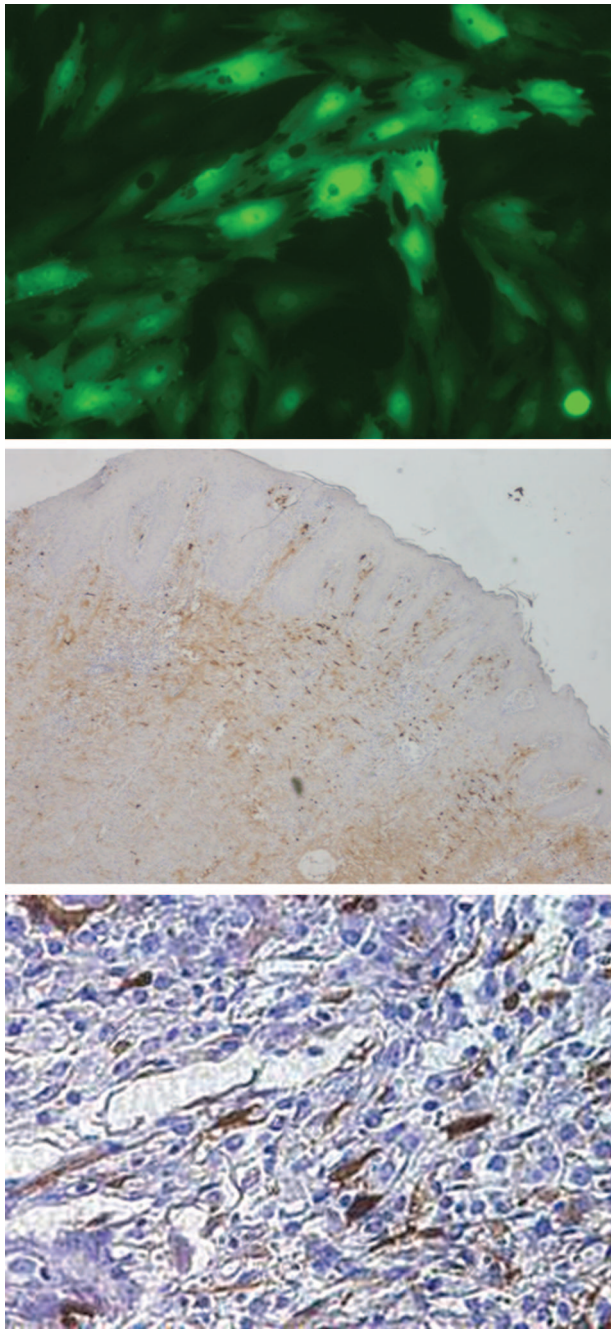


Fig. 6. Green fluorescence protein–adipose stem cell expression in dermal tissue. (Above) Fluorescence image of porcine-derived green fluorescence protein–adipose stem cells (original magnification, $\times 600$). (Center) Photomicrograph of a thin section from a 21-day biopsy specimen from a green fluorescence protein–adipose stem cell-treated wound site. Immunohistochemical staining shows widely distributed adipose stem cells (brown) within the papillary and rete pegs (black arrow) of the newly formed dermis. This region contains the microvascular network of the skin (original magnification, $\times 100$). (Below) Higher magnification of the image shown in center shows that green fluorescence protein–adipose stem cells (red arrow) adopt a perivascular location near a vessel denoted by the thick arrow (original magnification, $\times 1000$).

tein plus adipose stem cells in platelet-poor plasma at 21 days indicated that no apparent host rejection response occurred, as judged by a lack of migratory cell infiltration in thin sections. Direct visualization of green fluorescent protein expression in dermal tissues by fluorescent microscopy was not possible because of background autofluorescence of connective tissues; therefore, a mouse monoclonal antibody to green fluorescent protein was used for immunohistological detection in tissues. Based on hematoxylin and eosin staining, the green fluorescent protein–expressing adipose stem cells were found throughout the newly formed dermal layer, evenly spaced in the rete pegs and predominantly in perivascular locations (Fig. 6, center and below). These data suggest that adipose stem cells may have participated in revascularization by either recruiting endothelial cells to newly formed dermal tissue or by participating directly in vessel stability through associating with nascent capillaries, thus exhibiting pericyte functions.¹¹

DISCUSSION

This study demonstrates the potential for using multipotent adipose stem cells to promote wound healing. This effect was most evident when the adipose stem cells were supplied in combination with factor-rich platelet-rich plasma fibrin matrix. Evidence suggests that an important component of the wound-healing effect induced by adipose stem cells plus platelet-rich plasma was attributable to promoting enhanced vascularization of the repairing wound. Neovascularization is an important process in the healing of wounds, and it is possible that enhanced VEGF levels found when adipose stem cells were combined with platelet-rich plasma contributes to the higher content of arterioles formed in healed wounds treated with these agents. Clinically, it is well known that wounds with poor vascularity heal poorly. Studies in vascular disease patients have shown that large-vessel arterial bypass and improvement of lower limb blood flow improves wound-healing rates.¹²

The enhanced vascular density would seem to validate the evidence that adipose stem cells normally function in situ as pericytes to provide vascular stability and possibly to communicate with endothelial cells in response to environmental stimuli.¹¹ Adipose stem cells display cell markers associated with pericytes and secrete proangiogenic factors such as VEGF and human

growth factor and vessel-stabilizing factors such as angiopoietin-1. When provided with endothelial cells in vitro, adipose stem cells adopt a perivascular association and act to stabilize tube formation.

Even though adipose stem cells induce a robust angiogenic response in the healed wounds, a commensurate enhancement of the rate of reepithelialization was not observed, which is likely attributable to the inability to improve on the healing properties of normal, healthy skin. Presumably, an effect would be measured in compromised experimental systems possessing poorly vascularized tissues resulting from factors such as diabetes, age, or irradiation. It appears that the cell alone is not enough to improve healing.¹³⁻¹⁵

The quality of wounds was enhanced by application of adipose stem cells to the wound; however, the data suggest that complementary factors are required for this effect, because adipose stem cells suspended in platelet-poor plasma (fibrin glue) produced no improvement. Thus, data cannot be explained simply as confinement of adipose stem cells within the wound region. The platelet-rich plasma contains many factors indicated in wound healing, and we demonstrated that preparations from the blood of pigs contain both PDGF-BB and TGF- β . However, platelet-rich plasma alone as a delivery vehicle also did not improve outcomes. Nonetheless, when combined, adipose stem cells in platelet-rich plasma significantly improved wound healing. In particular, PDGF could be an important signaling ligand for adipose stem cells that express the PDGF receptor¹¹; thus, platelet-rich plasma may stimulate adipose stem cells to interact with and stabilize nascent vessels during ingrowth into the repairing wound.

Our findings support an innovative means of cellular therapy intervention to improve surgical wound healing in a normal wound model. Adipose stem cells supplied with platelet-rich plasma could be an efficacious and totally autologous therapy for treating surgical patients who have poorly healing wounds caused by vascular insufficiency, previous irradiation, or full-thickness burns. Because wound healing is a dynamic and complex process, the application of more than one growth factor with cellular therapy (adipose stem cells) demonstrates an advantageous way of improving healing. Complete understanding of these effects and validation of this new treatment modality will be possible through our current studies of these treatment combinations in an irradiated porcine wound model.

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