The Healing Effects of Autologous Platelet Gel on Acute Human Skin Wounds

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Objective: To compare the healing of full-thickness skin punch wounds treated with topical autologous platelet gel (APG) vs conventional therapy (antibiotic ointment and/or occlusive dressings) in healthy volunteers.

Methods: A prospective, single-blind, pilot study comprising 80 full-thickness skin punch wounds (4 mm diameter) was conducted on the thighs of 8 healthy volunteers. With each subject serving as his or her own control (5 punch sites per leg), APG was applied topically on one thigh, and an antibiotic ointment and/or a semiocclusive dressing was applied on the other thigh. Healing was monitored for spontaneous wound closure by clinical assessment and by digital photographs over 6 months. Over 35 days, 64 serial dermal biopsy specimens (6 mm diameter) were analyzed (using hematoxylin-eosin, Mason trichrome, CD-34, and Ki-67 stains) to measure differences between treated and control sites for cellularity, granulation formation, vascularity, epithelialization, and cellular replication.

Results: Over a 42-day period, the APG-treated sites had statistically increased wound closure compared with controls by visual clinical assessment and by digital planim-

etry photographic measurements ($P \leq .02$). On day 17, the percentage of closure was $81.1\% \pm 2.5\%$ (mean $\pm$ SE) for the APG-treated sites and $57.2\% \pm 5.9\%$ for the control sites. Also, the APG wound closure velocities were significantly faster than those of the controls ($P = .001$). Histologically, over time, the APG-treated sites had similar cellularity, cellular replication, granulation tissue, vascularity, and epithelialization compared with controls. However, when the platelet count in the gel was more than 6 times the baseline intravascular platelet count in some subjects, epithelialization and granulation formation appeared 3 days earlier in the APG-treated group. Furthermore, in vitro testing of supplemental APG showed increased endothelial cell proliferation compared with controls ($P < .04$).

Conclusion: This pilot study provides preliminary evidence that topical APG may hasten wound closure in full-thickness dermal wounds in healthy individuals.

Trial Registration: clinicaltrials.gov Identifier: NCT00199992

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INNOVATIVE DEVICES FOR PROCESSING autologous blood to concentrate platelet-rich plasma (PRP) into autologous platelet gel (APG) have recently become available.[1] Currently, APG is being used clinically in reconstructive, cosmetic, orthopedic, cardiovascular, oral maxillofacial, and dermatologic surgery in an attempt to improve tissue healing.[2-6] It is believed that platelets have concentrated levels of naturally occurring growth factors and other substances that have the potential to accelerate healing (**Table 1**). The use of APG to reduce ecchymosis and edema has received mixed reviews in clinical reports, and its clinical use remains controversial.[6-8]

To investigate whether topical APG can accelerate acute skin healing in healthy individuals, we conducted a prospective study testing APG on full-thickness skin punch wounds in healthy subjects. Full-

thickness dermal punch wounds were selected for the acute skin-healing model because the model is minimally invasive, technically straightforward to create, and easily followed up over time.[9,10] It also has minimal discomfort and low potential morbidity for subjects with reduced healing variability, which often limits healing measurements.[11] **Figure 1** schematically shows the healing steps of this full-thickness dermal wound model over time.

METHODS

After institutional review board review and approval, subjects were recruited on a voluntary basis to participate in the study. Volunteers eligible for the study were healthy men and women older than 21 years who were willing to follow instructions and be seen for 13 visits over 6 months. Informed consent, medical history, physical examination, and vital signs were

obtained. In the study, 80 full-thickness skin punch wounds (4 mm diameter) were made on the lateral thighs of 8 healthy volunteers (10 punch wounds per subject). In each subject, APG was applied to one thigh (5 punch sites), while the contralateral side (5 punch sites) served as the control. Therefore, each subject served as his or her own control to control for variables of nutrition, healing response, health status, and tissue oxygen level.

Individuals who were diabetic, were keloid or scar formers, had a collagen vascular disease or a bleeding disorder, or were taking an anticoagulant or a steroid medication over the last month were excluded from the study. Individuals who had an allergy to local anesthetic or bacitracin were also excluded. Women of childbearing potential had to have a negative pregnancy test result within 1 week of the study and were required to use a reliable method of birth control during the study.

PUNCH WOUND PROCEDURE

On day 0, the lateral aspect of the upper part of the thigh was shaved and disinfected with 70% alcohol and allowed to dry. After the administration of local anesthesia with 1% lidocaine, five 4.0-mm–diameter skin punch biopsies (Fray Products Corp, Buffalo, NY) were performed along the upper lateral thigh area (5 cm below the greater trochanter prominence) in a linear alignment (3 cm apart). Wound sites on each leg were labeled A, B, C, D, and E. Site E was the most proximal site on the leg. Hemostasis was obtained with 10 minutes of pressure to avoid cautery. The patients were seen in follow-up on days 1, 7, 10, 14, 17, 21, 24, 28, 31, 35, and 42 and 6 months later. They were instructed regarding proper wound care, eg, how to keep the area clean, while the wounds healed by secondary intention or were closed with suture after biopsy. In phase 1, APG was applied 1 time, on day 0. In phase 2, APG was applied twice, on days 0 and 7 (**Table 2**).

APG PREPARATION

To prepare the APG, two 60-mL aliquots of anticoagulated blood (13% anticoagulant citrate dextrose formula A) were obtained from each subject by venipuncture before the punch biopsies were performed. Each aliquot was processed by an autologous platelet separator (Magellan Autologous Platelet Separator; Medtronic Inc, Minneapolis, Minn) to yield 5 mL of PRP from each aliquot, thereby obtaining a total of 10 mL of PRP from each subject. One milliliter of PRP was used for platelet cell count analysis (Cell Dyn 1700 Hematology Analyzer; Abbott Diagnostics, Abbott Park, Ill) (**Figure 2**). An autologous serum dispenser kit (Magellan Autologous Serum Dispenser Kit; Medtronic Inc) was used to create approximately 1.3 mL of autologous thrombin-rich serum from 2 mL of PRP. The APG was created at the wound site by codispensing the remaining PRP and the thrombin-rich serum using an autologous serum dispenser kit and a 5-cm cannula tip (Magellan 2″ Cannula Tip; Medtronic Inc).

PHASE 1 (GROUPS 1 AND 2)

On day 0, APG was applied topically to the tested skin punch biopsy site, and the control received bacitracin and/or a semiocclusive dressing. Baseline platelet counts were obtained on all of the blood samples before autologous blood processing and after APG preparation.

Group 1

Autologous platelet gel (0.2 mL) followed by white petrolatum (USP) ointment (Topco Associates, Skokie, Ill) was ap-

Table 1. Substances Released From the α Granules of Platelets During Wound Healing

Platelet-derived growth factor
Basic fibroblast growth factor
Vascular endothelial growth factor
Transforming growth factor β1
Transforming growth factor α
Epidermal growth factor
Thrombospondin
Platelet thromboplastin
Coagulation factors
Serotonin
Histamine
Platelet-activating factor
Hydrolytic enzymes
Endostatin (antiangiogenic)

plied topically to each treated site. For the control group, a topical antibiotic (500 U/g of bacitracin zinc ointment [USP]; Walsh Dohmen Southeast, LLC, Birmingham, Ala) was applied to the control wounds. All wounds were subsequently covered with a semiocclusive dressing (Tegaderm Coverlet; Beiersdorf-Jobst Inc, Rutherford College, NC).

Group 2

Autologous platelet gel (0.2 mL) followed by semiocclusive dressing was applied topically to each treated site for 7 days. For the control group, the same semiocclusive dressing was applied to the control wounds for 7 days.

PHASE 2 (GROUPS 3 AND 4)

Phase 2 was identical to phase 1; however, in addition, APG (0.2 mL) was applied to each treated punch wound site for a second time on day 7. On days 0 and 7, 120 mL of blood was drawn and centrifuged with the autologous platelet separator to obtain 10 mL of PRP, and 2 mL of the PRP was used to make 1.3 mL of APG in the same manner as in phase 1. The skin biopsies in phase 2 were performed later because a second APG dose was administered on day 7. Group 3 was similar to group 1 and different only in that APG was applied twice. Group 4 was similar to group 2 and only different in that APG was applied twice.

MEASUREMENT OF HEALING PARAMETERS

In phases 1 and 2, the following wound healing parameters were measured and recorded on days 1, 7, 10, 14, 17, 21, 24, 28, 31, 35, and 42:

1. The remaining open wound area was measured at each punch site.

2. The time required for full closure of the dermal punch biopsy wound by secondary intention was determined.

3. In phase 1, on days 7, 10, 14, and 35, a second set of paired full-thickness skin punch biopsy specimens (6 mm diameter) (APG treated vs control) were obtained after a local anesthetic (1% lidocaine) was administered, and the incisions were closed with 3-0 nylon suture (at sites A, B, C, and D on each leg). The 6-mm-diameter skin specimens were placed in 10% buffered formalin for later histologic analysis. In phase 2, on days 10, 14, 17, and 35, a second set of paired full-thickness skin punch biopsy specimens (6 mm diameter) were obtained (at sites A,
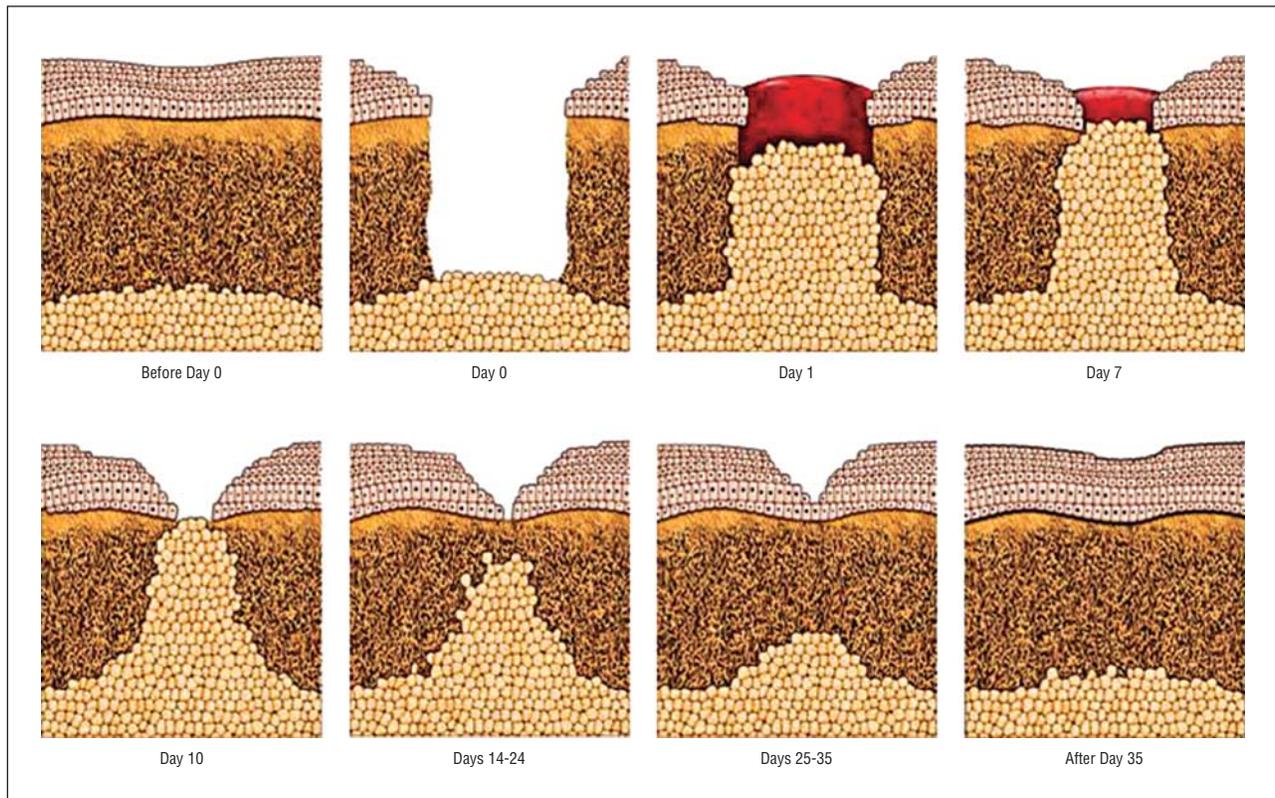
Figure 1. Schematic drawing of the skin wound healing model over time. On day 0, a full-thickness skin punch wound was created, resulting in adipose tissue prolapsing to the skin surface. On days 14 through 24, an epithelial and granulation tissue bridge formed. On days 25 through 35, the wound closed by secondary intention.

Table 2. Study Design of the Groups
Phase 1: APG applied topically as a 1-time dose to a 4-mm–diameter skin punch biopsy site
Group 1: APG (1-time dose) with white petrolatum ointment vs bacitracin ointment
Group 2: APG (1-time dose) with semiocclusive dressing vs semiocclusive dressing alone
Phase 2: APG applied topically as a 2-time dose (second dose given 7 d later) to a 4-mm–diameter skin punch biopsy site
Group 3: APG (2-time dose) with white petrolatum ointment vs bacitracin ointment
Group 4: APG (2-time dose) with semiocclusive dressing vs semiocclusive dressing alone

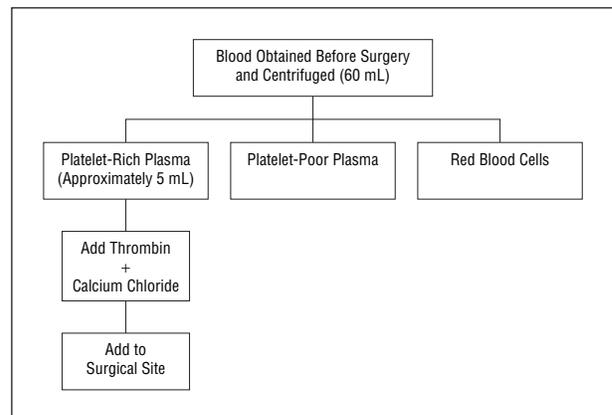Abbreviation: APG, autologous platelet gel.

Figure 2. Autologous platelet gel preparation.

B, C, and D on each leg) and then prepared and analyzed by the same methods as in phase 1.

At 6 months, the remaining punch wound sites (site E) that did not undergo biopsy were evaluated clinically to assess for scar size, color, and contour after healing by secondary intention.

TIME REQUIRED FOR COMPLETE WOUND CLOSURE

Clinical assessment was performed and standardized wound photographs were taken with a digital camera (Olympus 3040; Olympus, Melville, NY) with an adapter attachment through an optical microscope (OPMI 1; Carl Zeiss, Jena, Germany) at all sites on days 1, 7, 10, 14, 17, 21, 24, 28, 31, and 35 and

after 6 months. The photographs were later evaluated by blinded observers, who determined and recorded the time to achieve full closure. For statistical comparisons between treated and control sites, analysis of variance with repeated measures was used.

HISTOLOGIC MEASUREMENTS

Four serial biopsies (6 mm diameter) on sites A, B, C, and D were performed on each leg (treated and control sites) on days 7, 10, 14, and 35 in phase 1 and on days 10, 14, 17, and 35 in phase 2. The biopsy specimens were fixed in 10% buffered formalin for at least 24 hours. They were then embedded in paraffin and prepared in 4×6-μm transverse paraffin sections and

mounted on slides for evaluation of the following parameters under various stains: degree of angiogenesis on CD-34 stain; degree of cellular replication on Ki-67 stain; connective tissue production and turnover on Mason trichrome stain; and epithelialization on hematoxylin-eosin stain.

The extent of angiogenesis and connective tissue present was assessed by the amount of specific stain seen under high ($\times 400$) and low ($\times 20$) power in representative areas and scored in a blinded fashion by a histopathologist on a scale of 1 though 4: 1, no staining seen; 2, minimal staining seen; 3, moderate staining seen; and 4, excessive staining seen.

APG GROWTH FACTOR PROFILE

To investigate the change in growth factor levels in the preparation of PRP for APG, enzyme-linked immunosorbent assay kits (Quantikine Immunoassay Kit; R&D Systems, Minneapolis, Minn) were used to measure and compare the differences between growth factor concentrations in the initial whole blood samples and the resulting PRP used for the preparation of APG. Briefly, the steps of the technique were as follows: Initial blood samples from 9 different healthy volunteers were taken and centrifuged at 200g for 15 minutes to separate out the red blood cells, while the PRP samples were centrifuged at 150g for 5 minutes to remove any remaining red blood cells. The clear supernatants containing the platelets were treated with mammalian protein extract reagent (M-PER; Pierce Biotechnology, Rockford, Ill) to lyse all platelets and to cause them to release their growth factors. The resulting suspension was centrifuged at 14 000g for 15 minutes to remove cellular debris. The supernatant was used to assay for the growth factor of interest using the 96-well plate provided in the kit with a microtiter plate reader (SpectraMax; Molecular Devices Corp, Sunnyvale, Calif). Tumor growth factor β1 required an activation step before it was assayed in the 96-well plate. Activation of latent tumor growth factor β1 was achieved by the addition of a mixture of 2.5N acetic acid and 10M urea. The reaction was stopped after 10 minutes by reversing the acidified samples with a mixture of 2.7N sodium hydroxide and 1M HEPES (*N*-2-hydroxyethylpiperazine-*N′*-2-ethanesulfonic acid).

EFFECT OF APG ON ENDOTHELIAL CELL REPLICATION IN VITRO

To determine the effect of APG on endothelial cell replication, a cell proliferation assay (CellTiter 96; Promega Corp, Madison, Wis) was performed using human microvascular endothelial cells derived from the dermis. The number of cells was counted at 24, 48, 72, and 96 hours. The APG group was compared with controls consisting of basal medium, basal medium with serum growth factors, basal medium with platelet-free plasma gel, and basal medium with thrombin.

The steps of the technique were as follows: Thrombin was added to PRP in 3 wells of a 48-well plate to form APG in the APG-treated group. Similarly, thrombin was added to platelet-free plasma to form platelet-free plasma gel. The human microvascular endothelial cells derived from the dermis were trypsinized, resuspended in microvascular endothelial cell medium 2 (Clonetics EGM-2MV; Cambrex BioScience, Walkersville, Md), and then counted using a trypan blue exclusion. A total of 3750 cells were added to each well and incubated at 37°C and 5% carbon dioxide for 4 hours to facilitate adhesion of the cells to the culture plate. For each condition, 3 replicate wells were used. After the incubation period, the EGM-2MV was removed from all the wells and the wells were rinsed twice with Hanks balanced salt solution. Basal medium 2 (EBM-2; Cambrex BioScience) was added to all the rinsed wells except for the group

that received basal medium with serum growth factors (EGM-2MV). The plate was placed in an incubator at 37°C and 5% carbon dioxide until the number of cells was to be counted.

Analysis of variance with repeated measures was used to determine whether there were statistical differences between treatments at each time point. *P* values between specific treatment pairs were calculated using *t* tests.

RESULTS

The study included 4 men and 4 women (age range, 21-58 years). Over the 6-month course of the study, there were no dropouts and every subject went to every follow-up visit (12 follow-up visits per subject, for a total of 96 follow-up visits for all subjects). Among the 8 subjects, who had 80 dermal punch wounds, no infections were evident. The patients tolerated the APG treatment well, with no serious adverse events. On day 0, 1 patient had persistent oozing on 1 control skin punch site and required brief electrical cauterization for hemostasis. Sixty-four serial skin biopsy specimens were obtained serially from the 80 full-thickness punch wounds for histologic analysis, and the remaining sites were monitored for spontaneous wound closure by secondary intention (site E). The advantages of this skin punch model were that each subject served as his or her own control and the sites were easily accessible for measurements and photographic analysis over 6 months.

WOUND CLOSURE MEASUREMENTS OVER TIME

According to visual clinical measurements of wound closure over a 42-day period, the APG-treated sites had statistically increased wound closure compared with the control sites ($P<.001$, using analysis of variance with repeated measures) (**Figure 3**). On day 7, the percentage of closure was 14.0%±1.1% (mean±SE) for the APG-treated sites and 7.0%±1.1% for the control sites. On day 14, the percentage of closure was 73.9%±2.9% for the APG-treated sites and 49.6%±3.6% for the control sites. On day 17, the percentage of closure was 81.1%±2.5% for the APG-treated sites and 57.2%±5.9% for the control sites by simply pooling data from all available sites at each visit (**Figure 4**).

According to digital planimetry photographic measurements, the APG-treated sites had significantly increased wound closure compared with control sites over a 42-day period ($P=.02$, analysis of variance with repeated measures). In matching the visual clinical wound closure assessments with the digital photographic planimetry measurements, a strong correlation of these measurements was evident (correlation coefficient ≥.90) (**Figure 5**).

TIME REQUIRED FOR COMPLETE WOUND CLOSURE

Regarding the specific time required to achieve complete wound closure, the APG-treated wounds had a tendency to have a higher percentage of complete closure compared with the control wounds on days 24 and 28 by clinical assessment and by photographic digital
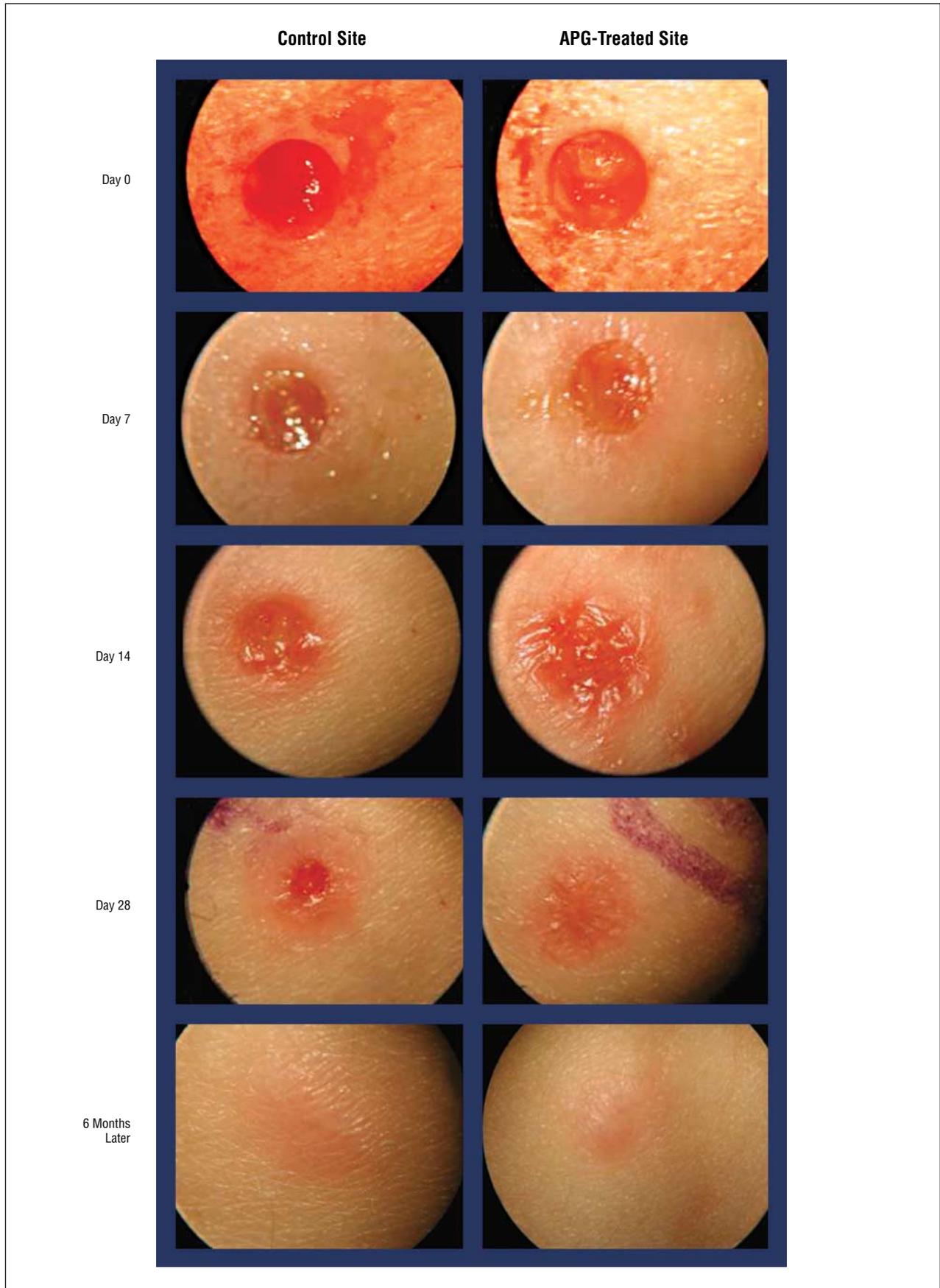
Figure 3. Clinical photographs of a representative wound site allowed to heal by secondary intention over time, showing the differences between the control site and the autologous platelet gel (APG)-treated site (site E) in subject 1 on days 0, 7, 14, and 28 and after 6 months.
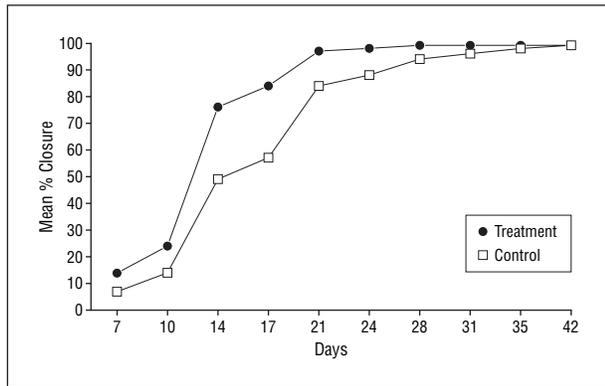
Figure 4. Clinical assessment of wound closure over time (sites D and E). The autologous platelet gel (APG)-treated sites had increased wound closure compared with the control sites over a 42-day period (*P*<.001). Specifically, on day 14, the percentage of closure for the APG-treated sites was 80% compared with 50% for the control sites.
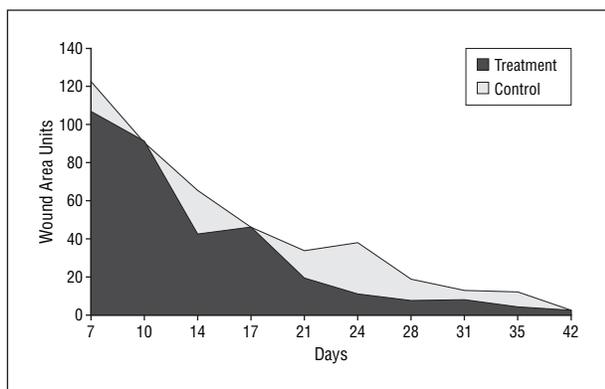

Figure 5. Total open wound surface areas of autologous platelet gel (APG)-treated sites vs control sites measured by digital planimetry over time. The APG-treated sites had a steeper slope (closure rate) between days 10 and 14.

measurements. Specifically, on day 21, 10 (63%) of the 16 APG-treated sites had full closure compared with 5 (31%) of the 16 control sites (*P*=.13, χ² test). On day 24, 13 (81%) of the APG-treated sites had full closure compared with 7 (44%) of the control sites (*P*=.07, χ² test). On day 28, 14 (88%) of the APG-treated sites had full closure compared with 9 (56%) of the control sites (*P*=.06, χ² test). However, because of the small sample size of each subgroup on each day, the *P* values were not significant (**Figure 6**). Among the punch wound sites that did not undergo biopsy (site E), the average time for the APG-treated wounds to achieve 100% closure was 29.75 days compared with 35.38 days for the control wounds.

HEALING CLOSURE VELOCITY

To quantify the wound healing closure velocity from day 0 to day 35 from the planimetry measurements, a biostatistician used a quantitative model that was based on an earlier proposed wound healing trajectory model for skin wounds. The wound healing trajectory approximately follows an exponential course.[12] Thus, the wound healing closure velocity from day 0 to day 35 from the planimetry measurements was modeled with an exponential decay formula with a single time constant as follows: $f(t)=A*exp$

Figure 6. Percentage of wounds that reached full closure according to planimetry measurements (by days).
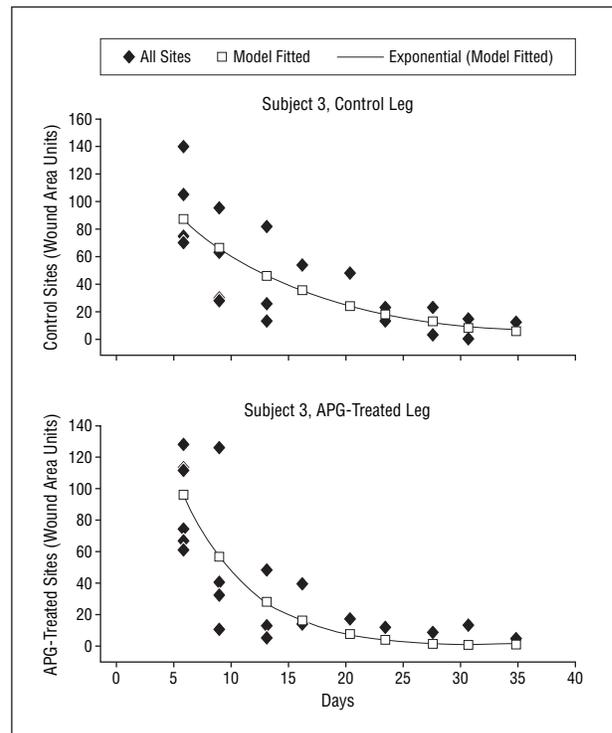

Figure 7. Wound healing trajectory depicted by the wound closure velocity in subject 3. The top graph (control wounds) shows the control data points of the open wound area over time (diamonds). The curved line with squares depicts the calculated wound healing closure velocity. The bottom graph (autologous platelet gel [APG]-treated wounds) shows the APG open wound area sites over time (diamonds) in the same subject, with the calculated wound healing closure velocity curve (squares).

$(-\lambda*t)$, where *A* is the initial open wound area, *exp* is the exponential function, λ is the rate of wound closure correlating with the slope of the curve, and *t* is the time in days. This formula correlated well with the closure rates seen for the control and treated sites over time. It incorporated all open wound area data points for a patient-treatment combination. **Figure 7** shows an example of a healing trajectory curve drawn from the exponential decay formula from all data points of the open wound area in 1 subject. Using this formula, the calculated healing curve closely matched the wound closure rate.

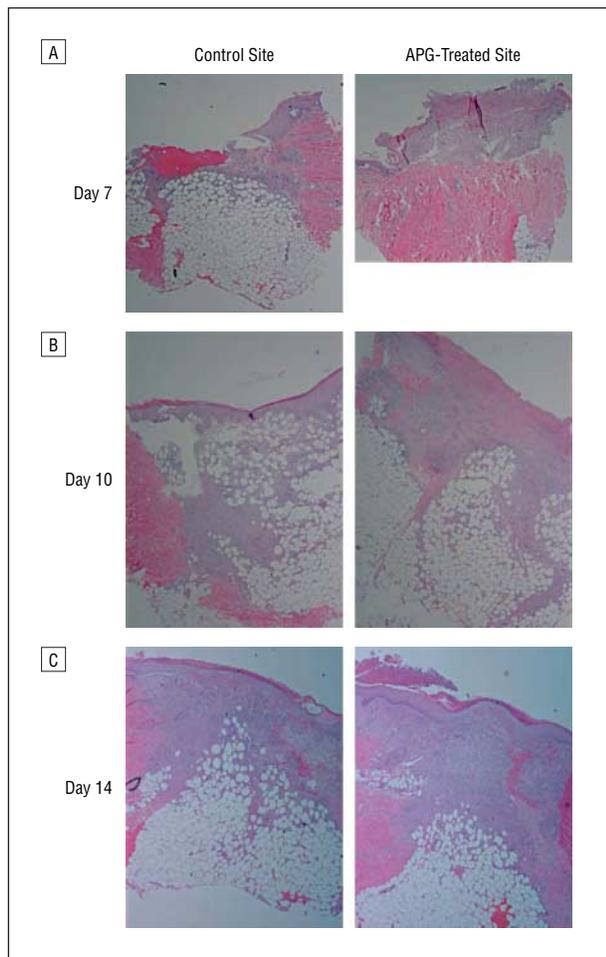The APG-treated sites appeared to heal faster than the control sites (with the APG-treated sites showing a steeper

Control Site   APG-Treated Site

Day 7

Day 10

Day 14

Figure 8. Subject 3. Clot formation and epithelial encroachment over the wound on day 7 (A), granulation tissue bridge formation over the wound on day 10 (B), and formation of a thicker granulation tissue bridge over the autologous platelet gel (APG)-treated wound on day 14 (C) (hematoxylin-eosin, original magnification ×20).

Control Site   APG-Treated Site

Day 14

Figure 9. Subject 3. A slide similar to that seen in Figure 8C shows mature (dark blue) and immature (light blue) collagen in granulation tissue bridge forming over the wound on day 14 (Masson trichrome, original magnification ×20).

Table 3. Growth Factor Assays (ELISA)*

Growth Factors	Initial Blood Sample (60 mL)	PRP (6 mL)
PDGF-AB, ng/mL	10.2 ± 1.4	88.4 ± 28.8
PDGF-AA, ng/mL	2.7 ± 0.5	22.2 ± 4.2
PDGF-BB, ng/mL	5.8 ± 1.4	57.8 ± 36.6
TGF-β1, ng/mL	41.8 ± 9.5	231.6 ± 49.1
VEGF, pg/mL	83.1 ± 65.5	597.4 ± 431.4
bFGF, pg/mL	10.7 ± 2.9	48.4 ± 25.0
EGF, pg/mL	12.9 ± 6.2	163.3 ± 49.4

Abbreviations: APG, autologous platelet gel; bFGF, basic fibroblast growth factor; EGF, epidermal growth factor; ELISA, enzyme-linked immunosorbent assay; PDGF, platelet-derived growth factor; PRP, platelet-rich plasma; TGF-β1, transforming growth factor β1; VEGF, vascular endothelial growth factor.

*Values are given as mean ± SD (adapted with permission from Medtronic Inc, Minneapolis, Minn). Blood samples were obtained from 9 healthy subjects (different volunteers from outside the wound punch study); APG was made by combining PRP with thrombin.

healing slope, with a qualitative shift of the curve to the left, compared with the control sites). This faster healing curve was most pronounced before day 14. In looking at the overall healing velocity and incorporating all time points by comparing the decay parameters obtained from the exponential formula between the APG-treated sites and the control sites, the treated sites had a statistically significant faster healing velocity than the control sites (paired t test, $P = .001$). Thus, the rate of wound closure was especially faster in the APG-treated sites compared with the control sites within the first 14 days.

HISTOLOGIC FINDINGS

Histologically, some early qualitative trends were seen in the dermal biopsy specimens when the platelet count in the gel concentrate was more than 6 times the baseline intravascular platelet count. In some subjects who had a more than 6-fold increase in platelet count in the gel concentrate, the APG-treated site showed more epithelialization over the adipose tissue on day 7 than its matched-paired control site (**Figure 8**A). By day 10, more granulation tissue was also present earlier in the APG-
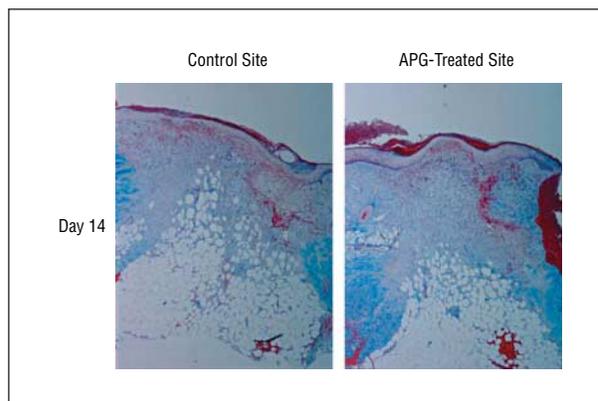
treated sites than in the control sites (Figure 8B). On day 14, a thicker granulation tissue bridge was present in some of the APG-treated sites (Figure 8C and **Figure 9**). On day 17, both control and APG-treated sites were very similar in the amount of epithelialization and granulation tissue that was present. In evaluating cellular replication by Ki-67 staining and new vessel growth by CD-34 staining at the peripheral edge of the wound on day 17, the treated and control groups were found to be similar histologically.

APG GROWTH FACTOR PROFILE ANALYSIS

The enzyme-linked immunosorbent assay was used to measure growth factor concentrations in initial blood and PRP samples from 8 different healthy volunteers. **Table 3** shows a 4-fold increase in basic fibroblast growth factor (FGF) levels to a 12-fold increase in epidermal growth factor levels after PRP preparation. Thrombin and PRP are combined to make the APG.

It was noted that the concentrations of any given growth factor in the platelets in the initial blood samples varied from

donor to donor. Also, the relative concentrations of the various growth factors (growth factor profile) within a donor varied across the donor population. On average, it was observed that the growth factor concentrations in the samples increased as the number of platelets increased, although variability was high in some cases. Thus, irrespective of the growth factor concentrations in the initial blood sample from a given donor, there was at least a 4-fold increase in the growth factor concentration in the APG that was prepared using the PRP obtained.

EFFECT OF APG ON ENDOTHELIAL CELL REPLICATION IN VITRO

Using the endothelial cell proliferation assay on human microvascular endothelial cells derived from the dermis, a statistically significant increase in endothelial cells cultured in the presence of APG was seen at 48, 72, and 96 hours later in comparison to their respective controls of basal medium, basal medium with serum growth factors, basal medium with platelet-free plasma gel, and basal medium with thrombin (P=.05 at 24 hours; P=.04 at 48 hours; P=.03 at 72 hours; and P=.01 at 96 hours) (**Figure 10**).

The basal medium served as a negative control, while the basal medium with serum growth factors was the positive control based on well-established cell culture techniques. In the case of the negative control, it was observed that cells initially proliferated, probably because of their exposure to serum growth factors before the experiment (cells were not serum starved before the experiment). However, over 72 and 96 hours, the basal medium alone was not sufficient to sustain the cells, and their number decreased. In contrast, the addition of serum growth factors in the positive control was sufficient to maintain the cell count over the entire 96-hour period. Because the APG provides a 3-dimensional matrix into which the endothelial cells can potentially proliferate, the platelet-free plasma gel was used to assess the 3-dimensional effect of the gel and the contribution of the free plasma levels of growth factors. Therefore, the difference between the cell counts in the presence of APG and the counts in the presence of platelet-free plasma gel could be attributed to the growth factors provided by the activated platelets in the APG. The basal medium with thrombin group showed that the addition of thrombin alone did not have a positive effect on endothelial cell proliferation.

COMMENT

The purpose of this pilot study was to compare the healing differences of full-thickness skin punch wounds with topical APG and conventional therapy in healthy volunteers. We found that APG-treated sites showed improved healing over a 42-day period compared with conventional therapy. Specifically, by visual clinical measurements over 42 days, APG-treated wounds demonstrated higher wound closure rates than control wounds. These clinical findings strongly correlated with the separate blinded digital planimetry photographic measurements. Also, the wound healing closure velocity
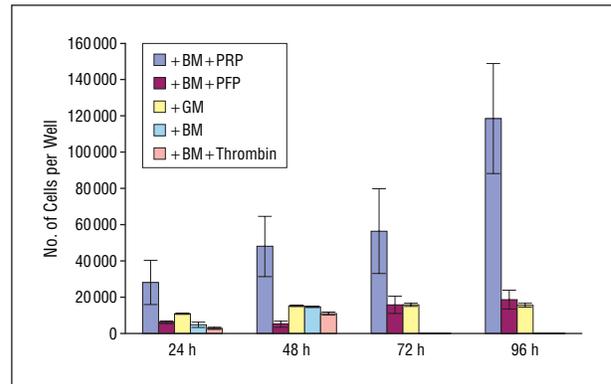

Figure 10. Endothelial cell proliferation assay on human microvascular endothelial cells derived from the dermis at 24, 48, 72, and 96 hours (adapted with permission from Medtronic Inc, Minneapolis, Minn). Autologous platelet gel (APG) was compared with controls consisting of basal medium without serum growth factors (BM), BM with serum growth factors (GM), BM with platelet-free plasma (PFP), and basal medium with thrombin; APG was made by combining platelet-rich plasma (PRP) and thrombin; and PFP was made by combining PFP and thrombin. At 48, 72, and 96 hours, the differences were statistically significant (P=.05 at 24 hours; P=.04 at 48 hours; P=.03 at 72 hours; and P=.01 at 96 hours). Error bars represent SD.

within 14 days of wounding was faster in the APG-treated sites than in the control sites.

When the platelet count in the gel concentrate was more than 6 times the baseline intravascular platelet count in a subgroup of subjects, APG appeared to have a greater histologic effect by accelerating epithelialization and granulation tissue formation. This histologic finding may be because a higher concentration of growth factors was more available to influence healing from the higher number of platelets delivered. Histologically, it appears that APG had its greatest effect on healing early on (<14 days after wounding), and by 17 days, the control wound caught up in the histologic analysis. This finding correlates with a recent study on a rodent model showing that the effect of platelet gel on healing appears to occur in a transient fashion within 14 days of administration.[13] Our preliminary findings indicate that APG has the potential to improve healing in acute full-thickness dermal wounds in healthy subjects. These findings are consistent with earlier reports suggesting that platelet releasate may actively promote acute dermal wound healing.[2,14]

What are the possible mechanisms of enhanced healing of APG in full-thickness skin punch wounds? One explanation is that both granulation tissue formation and epithelialization were enhanced by APG. This enhancement was histologically observed in some subjects when the platelet levels in the gel concentrate were more than 6-fold higher than the intravascular platelet levels. Another explanation is that wound contraction was enhanced by APG, allowing faster wound closure. Previous studies have reported that platelet-derived growth factor and transforming growth factor β1 (both of which are found in APG) can transform fibroblasts into myofibroblasts and cause myofibroblasts to contract.[15,16] Also, wound contraction could be enhanced by calcium, which is added during the platelet gel preparation. Further investigations are required to determine whether these mechanisms play a role in improved healing.

COULD APG BE USEFUL
IN ACUTE SURGICAL WOUNDS?

The long-standing goal in surgery is to achieve optimal wound healing after surgery. Through the ages, it has been generally assumed that normal acute wound healing is the best outcome that can be expected. However, is it possible to make normal wounds heal faster? We realize that attempting to improve the normal healing of healthy subjects remains controversial. One point of view may ask, "If a normal wound is going to heal anyway, what is the point of accelerating it?" There are at least 2 possible answers to this question:

1. "If it is possible to hasten normal wound repair, it should improve quality of life in surgical patients during their postoperative recovery." In fact, a previous study has suggested that acute wound healing can be enhanced by growth factor therapy. In a clinical study by Cohen and Eaglstein,[11] topical application of a single isolated growth factor, recombinant human platelet-derived growth factor (becaplermin), was shown to enhance the closure of acute dermal wounds in healthy subjects. Therefore, using growth factors to make normal wounds heal faster is an interesting possibility and could change the way we manage normal surgical wounds.

2. "It may be theoretically possible to prevent problem wounds from developing in postoperative patients by the administration of APG during surgery to those who are prone to poor healing." This prophylactic use of APG would be a scenario similar to administering prophylactic antibiotics in the head and neck region in a clean-contaminated surgical case to reduce the risk of postoperative wound complications from infection.[17] The prophylactic use of growth factors to prevent tissue injury has shown some promise in both animal and human studies. In the FGF family, basic FGF (FGF-2) was shown to reduce radiation injury and to reduce adverse postsurgical healing in irradiated skin in the porcine model.[18] In humans, keratinocyte growth factor 2 (palifermin) is a recent FDA-approved product that is used to reduce the severity and duration of oral mucositis in patients who receive chemoradiation therapy for hematologic cancers.[19] Thus, a prophylactic approach using growth factors to prevent wound complications is an intriguing possibility that could alter how we manage compromised surgical wounds in the future.

COULD APG BE EFFECTIVE
IN CHRONIC WOUNDS?

If specific growth factors are deficient or dysfunctional in chronic wounds, could adding APG improve chronic wound healing? The following characteristics make a chronic wound different from an acute healing wound: chronic wounds are thought to have increased proteases, increased proinflammatory cytokines, decreased protease inhibitors, and decreased growth factors.[20] Also, FGFs and transforming growth factor β concentrations are down-regulated in chronic wounds and are significantly lower than those in acute wounds.[18,21] By adding APG as a growth factor source to the wound, the increased protease activity within the chronic wound would have to be counteracted to prevent the breakdown of the growth factor proteins contained in APG.

Over the last 15 years, clinical trials have studied the topical effects of exogenous recombinant growth factors on chronic extremity skin wounds due to diabetes and vascular insufficiency. Results of these clinical studies have been both promising and disappointing. Several studies have shown promise in using platelet releasate to improve the healing of diabetic neuropathic foot ulcers.[22] It is important to emphasize that wound healing is dependent not only on the growth factor environment, but also on nutrition, status of infection, wound care, and oxygen level of the tissues.[23,24]

Chronic nonhealing wounds are the result of multiple causes (eg, diabetes, radiation exposure, ischemia, persistent infection, and neoplasia), with each chronic condition having its own pathologic process. Thus, the treatment of each chronic condition should be tailored to achieve optimal therapy, eg, surgical debridement, moist dressings, control of infection, and proper nutrition. To improve the healing of chronic diabetic neurotrophic foot ulcers, the application of a single growth factor, platelet-derived growth factor, was most effective when used in conjunction with good wound care.[25] Therefore, growth factor therapy should be considered an adjunctive means of therapy and not a replacement for standard wound care.

LIMITATIONS

Several limitations of our study should be noted. This was a pilot study, and the APG application and initial clinical measurements were performed in a single-blinded fashion. However, the photographic planimetry data and histologic measurements were determined in a blinded fashion. Even so, the initial wound clinical closure measurements statistically correlated strongly with the blinded photographic planimetry data.

Another limitation of our study is that the platelet count in the gel concentrate varied among the subjects. When the platelet count in the APG was more than 6 times the subject's baseline platelet count, the beneficial effects of APG became more histologically apparent and consistent (5 of 8 subjects had platelet gel concentrates greater than 6 times the baseline intravascular platelet levels). Therefore, in the future, if consistently elevated levels of platelets greater than 6 times the baseline platelet count levels can be achieved by APG processing, more beneficial results in wound healing may be seen.

One concern with supplemental growth factors is that they could increase the risk of malignancy or excessive scarring. However, to our knowledge, no studies to date have reported any evidence of malignant transformation using supplemental growth factors when there is no preexisting genetic mutation. Also, we know of no clinical study in which growth factor therapy has resulted in an increased propensity to keloid or hypertrophic scar formation. In previous studies, the effects of supplemental growth factors seem to occur in a transient manner on their target cells.[20]

By increasing our understanding of APG on acute full-thickness skin wounds, new growth factor interventions may improve soft tissue healing in surgical patients. If APG treatment accelerates acute dermal healing, it could prevent and reduce some postsurgical wound healing complications commonly seen in patients who are susceptible to poor healing.

In this pilot study, APG appeared to enhance wound closure in acute full-thickness dermal wounds in healthy subjects. Furthermore, the wound closure velocity of APG-treated wounds was greater than that of control wounds. It appears that when the platelet count in the gel is more than 6 times the baseline intravascular platelet count, the effects of the gel are more pronounced histologically. Further investigations are needed to confirm the consistency of these results. If further studies support these findings, APG treatment during surgery could have a useful impact on the enhancement of postoperative dermal wound healing in surgical patients.

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