Platelet-rich plasma (PRP) or platelet releasates have been proposed as surgical adjuncts for the treatment of wound healing, including recalcitrant, severe leg ulcers, while reducing infections and length of hospital stay. In most applications, PRP is mixed with thrombin before clinical application as a means of generating a platelet gel (PG) biomaterial that is applied to tissues. The scientific rationale for the clinical use of PG is that thrombin-activated platelets release numerous growth factors (GFs) from their α-granules that can modulate cell proliferation and differentiation and accelerate soft tissue repair in vivo. Those include at least seven locally acting GFs: three isomers of platelet-derived growth factor (PDGF-AA, PDGF-AB, and PDGF-BB), two isomers of transforming growth factor-β (TGF-β1 and TGF-β2), vascular endothelial growth factor (VEGF), and epithelial growth factor (EGF). In an experimental model of laser-induced burns, autologous PG was also shown to favorably influence wound healing by stimulating an intense inflammatory process, leading to highly significant increases in the production of extracellular matrices and granulation tissue. PG may accelerate vascular ingrowth, increase fibroblastic proliferation, and accelerate collagen production. Such a highly vascularized bed may promote the success of skin grafting in patients with deep partial-thickness and full-thickness burns. Clinical studies using recombinant human (Rh) PDGF-BB (bepaclearmin) support the benefit of GFs in these indications. Rh-PDGF-BB was licensed more than 10 years ago for the treatment of neurotrophic diabetic ulcers. Randomized, prospective, multicenter trials demonstrated that once-daily topical application of Rh-PDGF-BB at a dose of 100 μg/g, in conjunction with good ulcer
care, could stimulate rapid healing of chronic neurotrophic ulcers of the lower extremity in patients with diabetes. PG and becaplermin appear more effective than standard care in improving healing rates in diabetic neuropathic foot ulcers at 20 and 32 weeks, becaplermin being more effective than PG after 20 weeks of care. Topical Rh-PDGF-BB was also found to be effective in the treatment of non-diabetic and non-pressure-related chronic ulcers. Finally, the use of fibrin glue (FG) (also known as fibrin sealant), obtained by mixing a fibrinogen-rich fraction with thrombin, has also been described to facilitate the fixation of skin grafts and limit the risk of infection in burn wounds.

In spite of this progress, chronic wounds often require skin grafts as the definite procedure for chronic wound healing. To the best of our knowledge, the use of PG to enhance the take of skin graft has not been documented. The purpose of this study was to evaluate the safety and efficacy of using PG and FG to enhance skin graft take for chronic lower extremity ulcers.

Materials and Methods

Patients

The Institutional Review Board of Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan, approved this study (Protocol 096-05-042). Eligible patients were enrolled after informed consent was obtained. The protocol conformed to ethical guidelines of the 1975 Declaration of Helsinki.

Preparation of Thrombin, PG, and FG

The Taiwan Blood Service Foundation (Taipei, Taiwan) prepared human fresh frozen plasma (FFP), platelet-rich plasma (PRP), and cryoprecipitate units from blood collected from volunteer non-remunerated donors, following the regulations of the Taiwan Department of Health. The blood bank of the Tri-Service Hospital (Taipei, Taiwan) thawed FFP and cryoprecipitate units at 30°C 1 hour before surgery, which were delivered, together with PRP, to the surgical suite. Thrombin was prepared as described before. Briefly, 10 mL of FFP and 0.3 mL of a 10% calcium chloride solution (Taiwan Biotech Co., Ltd, Taoyuan, Taiwan) were introduced into a sterile thrombin generation device (TGD-001; Merries International Inc., Shin Tien, Taiwan). The device was shaken gently for 30 seconds and then put aside to let the plasma activation reaction proceed at room temperature. After approximately 15 minutes, the thrombin-rich supernatant was drawn aseptically using a sterile 10-mL syringe. PG was obtained by spraying equal volumes (3 to 8 mL depending upon the surface of the wound) of PRP and thrombin using a spray applicator (Merries International Inc.) and FG by applying equal volumes (3 to 8 mL depending upon the surface of the wound) of cryoprecipitate and thrombin using a dual-syringe system (Merries International Inc.).

Study Design

This clinical study was a prospective pilot trial of patients with recalcitrant lower extremity ulcers. Patients were considered as candidates for the study if they had had recalcitrant ulcers not curable by traditional healing procedures for at least 3 months. Fifteen consecutive patients with 17 ulcers met these criteria and were treated from May 2007 to April 2008. There were five men and 10 women, aged 45 to 80. The characteristics of the patients (sex and age), medical history, and etiology of skin ulcers are reported in Table 1. Etiologies were venous insufficiency (7 ulcers), diabetes (4 ulcers), diabetes with uremia (3 ulcers), trauma (1 ulcer), burn (1 ulcer), and scleroderma (1 ulcer). The ulcers had a median size of 54.9 cm² (range 25–120 cm²). The median ulcer duration before being enrolled in this study was 14.2 months (range 3 months to 10 years).

The skin ulcers were first débrided to remove necrotic tissue. The wounds were covered with moist saline dressing. Daily dressing change without additional treatment was performed. Repeated débride-ment was necessary in five ulcers (ulcers 1, 2, 3a, 3b,
<table>
<thead>
<tr>
<th>Patient</th>
<th>Ulcer Number</th>
<th>Sex</th>
<th>Age</th>
<th>Cause of Ulcer</th>
<th>Duration of Ulcer</th>
<th>Size and Location of Ulcer</th>
<th>Prior Ulcer Therapy</th>
<th>Take of Skin Graft</th>
<th>Time to Healing</th>
<th>Follow-Up Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>F</td>
<td>80</td>
<td>Venous insufficiency</td>
<td>10 years</td>
<td>14 × 6 cm², left lower leg</td>
<td>Multiple skin grafts with failure</td>
<td>2 × 2 cm²</td>
<td>1 month</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>F</td>
<td>65</td>
<td>Diabetes with uremia</td>
<td>1 year</td>
<td>5 × 8 cm², right heel</td>
<td>Multiple débridements &amp; HBO</td>
<td>2 × 1 cm²</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>3a</td>
<td>F</td>
<td>55</td>
<td>Diabetes</td>
<td>8 months</td>
<td>6 × 20 cm², left lower leg</td>
<td>Multiple débridements</td>
<td>3 × 1 cm²</td>
<td>1 month</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>3b</td>
<td>F</td>
<td>55</td>
<td>Diabetes</td>
<td>8 months</td>
<td>5 × 20 cm², right lower leg</td>
<td>Multiple débridements</td>
<td>Complete</td>
<td>3 weeks</td>
<td>12</td>
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<tr>
<td>4</td>
<td>4</td>
<td>M</td>
<td>60</td>
<td>Burn</td>
<td>2 years</td>
<td>10 × 6 cm², right lower leg</td>
<td>Conventional dressing change</td>
<td>Complete</td>
<td>3 weeks</td>
<td>12</td>
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<td>5</td>
<td>M</td>
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<td>Scleroderma</td>
<td>3 months</td>
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<td>Conventional dressing change</td>
<td>Complete</td>
<td>3 weeks</td>
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<td>6</td>
<td>6</td>
<td>F</td>
<td>52</td>
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<td>15 × 7 cm², right lower leg</td>
<td>HBO</td>
<td>Complete</td>
<td>3 weeks</td>
<td>3</td>
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<tr>
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<td>7</td>
<td>M</td>
<td>56</td>
<td>Venous insufficiency</td>
<td>1 year</td>
<td>5 × 8 cm² right lower leg</td>
<td>HBO</td>
<td>Complete</td>
<td>3 weeks</td>
<td>12</td>
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<tr>
<td>8</td>
<td>8</td>
<td>M</td>
<td>52</td>
<td>Trauma</td>
<td>6 months</td>
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<td>Multiple débridements</td>
<td>Complete</td>
<td>3 weeks</td>
<td>3</td>
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<tr>
<td>9</td>
<td>9</td>
<td>M</td>
<td>55</td>
<td>Venous insufficiency</td>
<td>8 months</td>
<td>10 × 6 cm², right lower leg</td>
<td>Conventional dressing change</td>
<td>Complete</td>
<td>3 weeks</td>
<td>10</td>
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<tr>
<td>10</td>
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<td>F</td>
<td>56</td>
<td>Venous insufficiency</td>
<td>9 months</td>
<td>12 × 5 cm², right lower leg</td>
<td>Conventional dressing change</td>
<td>Complete</td>
<td>3 weeks</td>
<td>12</td>
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<tr>
<td>11</td>
<td>11</td>
<td>F</td>
<td>62</td>
<td>Diabetes with uremia</td>
<td>1 year</td>
<td>5 × 5 cm² left malleolus</td>
<td>HBO</td>
<td>2 × 1 cm²</td>
<td>1 month</td>
<td>15</td>
</tr>
</tbody>
</table>
11) because of residual necrotic tissue. The interval between the débridement and skin graft ranged from 3 to 14 days.

During the procedure, the wound bed was sprayed with PG, and a thinsplit-thickness skin graft with multiple slits was put on the wound bed. Finally, FG was sprayed on the skin graft; no sutures or staples were necessary. A short leg polypropylene splint was used to immobilize the skin graft.

Results

No treatment-associated adverse reactions were observed during the study. Most (13/17) of the skin grafts took well. Minor areas of skin graft loss, corresponding to less than 5% of the total skin graft area, were noted in four ulcers (2 ulcers caused by diabetes with uremia, 1 by diabetes, and 1 by venous insufficiency), which healed spontaneously using a conventional saline dressing. The interval between skin graft and complete wound healing ranged from 3 weeks to 2 months. No recurrence of ulcers was noted during the follow-up period, which ranged from 3 to 18 months. The most relevant cases are presented below. Patient 1 was an 80-year-old woman who presented with a chronic venous ulcer on the left lower leg for 10 years, refractory to multiple conventional skin grafts. The ulcer measured $14 \times 6 \, \text{cm}^2$, down to the periosteum of tibia, with soft tissue necrosis (Figure 1A). Débridement was performed twice to remove the necrotic tissue. Ten days after the second débridement, the wound bed was sprayed with PG (Figure 1B). A thin-split-thickness skin graft was then applied to the wound. Finally, FG was sprayed on the skin graft (Figure 1C), which took well. A minor area of skin loss ($\sim 2 \times 2 \, \text{cm}^2$) was noted, which healed spontaneously using a conventional saline dressing for 3 weeks. The postoperative course was uneventful, and the patient has durable wound coverage 18 months after skin graft (Figure 1D).

Patient 4 was a 65-year-old man who presented with chronic skin ulcer caused by burn injury for 2 years.
The ulcer measured $10 \times 6 \, \text{cm}^2$ (Figure 2A). Three days after adequate débridement, the wound bed was sprayed with PG (Figure 2B), and a thin-split-thickness skin graft was applied to the wound. Finally, FG was sprayed on the skin graft (Figure 2C), which took without any loss. The wound

**Figure 1.** Patient 1. (A) Chronic venous lower leg ulcer for 10 years, refractory to multiple conventional skin grafts. (B) After repeated débridements, the wound bed was sprayed with platelet glue. (C) Thin-split-thickness skin graft with multiple slits covering the wound bed; fibrin glue was sprayed on the skin graft. (D) Durable wound coverage 18 months later.

**Figure 2.** Patient 4. (A) Chronic lower leg burn ulcer for 2 years. (B) Three days after débridement, the wound bed was sprayed with platelet glue. (C) Thin-split-thickness skin graft with multiple slits covering the wound bed; fibrin glue was sprayed on the skin graft. (D) Durable wound coverage 12 months later.
healed completely 3 weeks after the skin graft. The postoperative course was uneventful, and the patient has durable wound coverage 12 months after the skin graft (Figure 2D).

**Discussion**

The results of clinical studies using PG to treat chronic skin ulcers are conflicting. Encouraging results when treating skin ulcers with autologous or allogeneic PG have been reported.1–4,8 It was found that patients with various skin ulcers treated with PG experienced quicker healing, although in other clinical studies, neither platelet products nor recombinant growth factors have exhibited any clear adjuvant effect on chronic venous ulcer healing.21,22 Chronic wound fibroblasts generally exhibit a poorer mitogenic response to PDGF-AA, PDGF-BB, basic fibroblast growth factor, TGFβ1, and EGF than normal or acute wound fibroblasts. High levels of proteases are found in wound fluid from chronic venous ulcers, impairing the healing process by degradation of exogenous added growth factors. The discrepancies reflect not only differences of technical protocols for preparing PG, but also the complexity of chronic ulcer healing. It is beyond the scope of this study to debate whether PG alone is effective for healing of chronic skin ulcers. From a clinical point of view, skin grafting is commonly required as a definite procedure for healing of chronic skin ulcers.

In this article, we present a new approach developed specifically for healing recalcitrant leg ulcers. A combination of three therapeutic components is used sequentially. First, after careful débride ment of the wound, PG, obtained by mixing PRP with an equal volume of human thrombin, is applied using a double-syringe device equipped with a spray applicator. Within 5 to 10 seconds after mixing, the fibrinogen present in the PRP polymerizes into a fibrin gel, leading to the formation of a PG that adheres to the wound. Second, a thin-split-thickness skin graft is applied to the wound, on top of the PG. Third, FG, obtained by mixing equal volumes of cryoprecipitate and human thrombin, is sprayed on the graft. The blood products were of allogeneic origin because some patients were too old or not healthy enough to donate blood and the production of cryoprecipitate to prepare enough autologous FG would require a large volume (200 mL) of plasma that is not easy to process into cryoprecipitate within hospitals. In this procedure, the mixing of human thrombin and PRP, a fraction with a 5 times greater platelet concentration than baseline values in whole blood,23 leads to an activation process that releases multiple GFs from the platelet α-granules.5 In wound healing, platelets play an essential physiological role because their α-granules are rich with GFs. Although concentrations of GFs depend upon several factors, such as the platelet preparation method24,25 and thrombin and calcium concentration,26 various systems for preparation of PRP result in a considerably greater release of GFs than levels in whole blood.27 In addition, blood banks preparing the platelet concentrates used should adhere to strict specifications in platelet count, thereby reducing the risk of variability in GF content. However, we cannot exclude that the amount of GF released varied somewhat, within a given range, between the various procedures described in this study. Several studies have shown that PDGF-AB and TGF-β1 are present in activated platelet releasates at 100 to 200 ng/mL,28–31 PDGF-BB at approximately 10 ng/mL,28 EGF and VEGF at 1 to 5 ng/mL, and TGF-β2 at approximately 0.5 ng/mL,28 whereas IGF-1, which circulates in plasma, is found at approximately 100 ng/mL.32 These GFs are important for re-epithelization and neovascularization by mesenchymal cell recruitment and extracellular matrix synthesis. The PG also induces little inflammatory reaction between the graft and the recipient area, resulting in favorable conditions for graft incorporation. Therefore, we postulate that it provides a favorable physiological environment for the success of the grafting procedure. The use of PG was found to enhance the take of the skin graft after adequate débridement of the wound. All of the skin grafts took without major loss, and the patients achieved durable wound healing in the follow-up period. Application of the FG, a product already
used for skin grafting and hemostasis,17–19 allowed more secure and stable placement of the skin graft, particularly because it could penetrate the thin-split-thickness skin graft and come into direct contact with the wound, contributing to the safety of the procedure. The procedure avoids the use of staples or sutures, which was of major benefit to patients because their removal is painful.

Although the clinical effectiveness of this study derived from a pilot study rather than from randomized controlled trials, the procedure provides useful advantages in skin grafting to treat recalcitrant ulcers because PG functions as a delivery system of powerful mitogenic and chemostatic factors and FG as a hemostatic tissue sealant.

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References


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