Platelet-rich plasma (PRP) is a platelet concentrate that has been widely used in a variety of clinical applications. A number of studies show that PRP enhances and accelerates both soft tissue and hard tissue healing.\(^1\)\(^-\)\(^3\) The effectiveness of PRP is based on its high level of growth factors such as platelet derived growth factor (PDGF), transforming growth factor-\(\beta\) (TGF-\(\beta\)), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and insulin-like growth factor (IGF), etc. These growth factors are important in modulating mesenchymal cell recruitment, proliferation, and extracellular matrix synthesis during the healing process.

Chronic cutaneous wounds are a frequent problem in developing countries and are often difficult to heal because they lack the necessary growth factors necessary for the healing process, and are frequently complicated by superinfection.\(^1\) Conventional therapies such as dressings, surgical debridement, and even skin grafting cannot provide satisfactory healing since these treatments are not able to provide enough necessary growth factors to modulate the healing process. Patients with chronic ulcers frequently have to undergo long-term dressing regimens and repetitive debridement without a definitive outcome. Some commercial recombinant growth factor products, like becaplermin (recombinant PDGF-BB), is FDA-approved for the treatment of chronic wounds, but its time-release delivery is a significant problem.
Figure 1. Before application of PRP, the wound was 6 cm in diameter.

Figure 2. After the first centrifugation, the whole blood was divided into three layers: red blood cells in the lowest level, platelets in the middle, and PPP at the top.

Figure 3. Drawing the red cells from the middle tube.

Figure 4. After the second centrifugation, the blood was divided into two layers: the upper was PPP, the lower was concentrate platelet.

Figure 5. A syringe with thrombin and 10% calcium chloride was fit together with the PRP syringe.

Figure 6. The wound was covered with PRP.

Figure 7. The size of the ulcer decreased significantly after the first application of PRP 2 weeks later.

Figure 8. Five weeks later, the size of wound continued to decrease.

Figure 9. A healed wound was achieved after three applications of PRP over 7 weeks.
because it is liquid and quickly disappears once it has been applied to the wound. Additionally, the product is expensive and most patients cannot afford such high cost in developing countries. Based on the authors’ clinical experiences, the use of recombinant growth factors may not be an ideal choice in the treatment of refractory ulcers.

PRP contains various growth factors that are necessary in wound healing. Moreover, it also has some other advantages. After blending with calcium and thrombin, PRP turns into gel, which prevents growth factors and leukocytes from releasing, and maintains their activity for a longer time within the wound. Additionally, a high concentration of leukocytes contained in PRP is also helpful in inhibiting infections.5

The preparation of PRP is simple. Only two centrifugations of autologous whole blood and a total of 20 to 30 minutes are required. PRP is biocompatible and safe, and does not carry an infection risk. We applied PRP gel on three patients with large ulcers. All 3 cases had a long history of conventional treatments without definitive results.

Case Reports

Case 1: A 53-year-old woman underwent a transperitoneal left renal cyst decompression operation. During the procedure, an electrical leakage of the metal plate electrode burnt the skin above the left medial malleolus and resulted in a necrosis with a diameter of 4 cm. Despite immediate oral antibiotic (cephalosporins) and regular dressing treatments, the wound gradually became larger and deeper and bony tissue finally became exposed. The wound diameter increased to 6 cm 1.5 months later (Figure 1). While she was receiving regular dressing treatments, a culture for Staphylococcus aureus returned positive. Fifty days later, the patient was admitted to our department and PRP therapy was initiated.

PRP preparation was performed in a sterile environment. Thirty milliliters of whole blood was drawn from the elbow vein. The syringe had been anticoagulated with acid citrate dextrose (ACD) with a ratio of 1:9 to the blood. After a 10-minute centrifugation with a force of 200 g, the blood was then layered into three basic components: red blood cells, platelets, and platelet-poor plasma (PPP). The red blood cell layer was at the lowest level because of different sedimentation coefficients, the platelet layer was in the middle, and the PPP layer was at the top (Figure 2). About four-fifths of the red cells were drawn from the middle tube (Figure 3). The remainder was agitated for several seconds and underwent a second centrifugation at 200 g for 10 minutes. The blood was then centrifuged into two layers; the supernatant was PPP while the lower layer was concentrate platelet (Figure 4). About three-quarters of the supernatant was discarded. The residual PRP (approximately 4 mL) was drawn into a syringe. The platelet concentrate in the PRP was measured to be 6 times the amount found in the baseline count of the whole blood. Another syringe with thrombin and 10% calcium chloride were integrated with the PRP syringe in order to inject PRP and thrombin simultaneously (Figure 5). After clearing the wound, PRP with thrombin/calcium chloride were applied to the ulcer (Figure 6). When PRP was sprayed onto the wound it transformed into a gel. A transparent membrane was used to cover the wound.

On the second day, after the first PRP application, the patient reported pain relief. Over the following 3 weeks and two PRP treatments later, the wound became smaller and granulation tissue grew intensively. The membrane was removed 2 weeks later and the wounds size had decreased significantly (Figure 7). A total of three applications of PRP were performed on this patient within 7 weeks before the wound healed completely (Figures 8, 9).

Case 2: A 41-year-old man fell from a 4-meter height 15 months earlier, which resulted in a second lumbar vertebrae fracture and paraplegia of the lower body. Seven months later, a decubital ulcer was noticed at the inferior surface of the right great trochanter of the femur. After regular wound dressing treatments and approximately 10 debridements, the ulcer grew in size and depth, and the amount of effusion increased significantly. When admitted to the authors’ hospital, the ulcer was 8 cm long and 7 cm wide with yellowish, necrotic tissue (Figure 10). The fistula below the wound extended upward to the superior anterior iliac spine and had a large, underlying dead space (Figure 11).

Simple debridement was performed to remove necrotic tissue before the first application of PRP. The same method was used to prepare the PRP. After the first application, the amount of effusion decreased significantly as observed through the transparent membrane. Wound dressing with PRP therapy was performed every 10 to 15 days. During this period of PRP treatment, the necrotic tissue disappeared gradually. At the third week, a considerable amount of granulation tissue was observed (Figure 12). Gradually, the granulation tissue had completely filled the whole underlying space by week 8 (Figures 13, 14). Ten weeks following admission,
suturing was performed to aid in wound skin contraction. After 12 weeks, the wound had healed almost completely (Figure 15). Fourteen weeks and 9 PRP applications later, the wound achieved complete healing (Figure 16).

**Case 3:** A 9-year-old boy injured himself after his left foot rolled into a bicycle wheel. The injury caused a large defect on the soft tissue of the heel in addition to exposed calcaneal bone. Some fractured bone particles were seen in the wound (Figures 17, 18). Traditional therapies including antibiotics, debridement, and regular dressing changes failed and the condition of the wound deteriorated. After admission to the authors’ hospital, it was also observed that the Achilles tendon was exposed. Previously, a skin flap transfer would have been considered as treatment. Considering the lower survival rate with such a wound, we attempted PRP treatment on this patient (Figure 19). After the first PRP treatment, the patient was told to follow up with the outpatient clinic 2 weeks later. Much to our surprise, at his first visit the wound had almost completely healed with only a minor skin defect remaining (Figure 20). Three days later, the wound was healed and had completely epithelialized (Figure 21).
Discussion

In all three cases excellent outcomes were achieved after topical PRP application. No adverse effects or complications have occurred thus far with PRP use. Previous conventional treatments, such as wound dressings and surgical debridement, proved ineffective in treating such refractory wounds. In these types of wounds, the balance between cytokines and extracellular matrix synthesis has been broken. The skin and extracellular matrix are difficult to regenerate and can lead to erosion and deterioration; therefore, primary fresh wounds progress into a chronic state. Thus, important growth factors and cytokines, which PRP contains, are required to stimulate and initiate the healing process.

Activated by thrombin and calcium chloride, PRP can release several critical growth factors. All of these platelet growth factors are crucial to wound healing. In our third case, the patient had plenty of mesenchymal cells in the tissues around the ulcer and the growth factors with high concentration in PRP stimulate proliferation and differentiation of these mesenchymal cells. The above mechanism may account for his rapid healing. According to the Marx et al study, young people have the superiority in the number of healing capable stem cells. Therefore, younger patients heal faster than elderly patients.

The anti-inflammatory factors in PRP also play an important role in wound healing. Leukocytes are also at high levels in PRP because the density of leukocytes is similar to that of the platelets, and after centrifugation they both drop into the same layer of the centrifuge tube. In particular, these leukocytes include neutrophils, granulocytes, and monocytes, all of which are known for their actions against bacteria through secretion of myeloperoxidase that creates hypochloric acid. El-Sharkawy et al found that PRP made significant changes in monocyte-mediated proinflammatory cytokine/chemokine release and increased the level of

![Figure 17. Large soft tissue defect of the heel.](image1)

![Figure 18. X-ray showed some fracture particles in the wound; the wound was deep and extended to the surface of the calcaneus.](image2)

![Figure 19. PRP was used to cover the wound.](image3)

![Figure 20. Fourteen days later, the wound was almost completely healed.](image4)

![Figure 21. Seventeen days later, the wound healed completely with complete epithelialization.](image5)
lipoxin A4, which limits inflammation and infection. Bielecki et al.\textsuperscript{10} reported that PRP gel inhibited the growth of \textit{S. aureus} and was also active against \textit{Escherichia coli}. In the first case, bacteriologic culture of the wound was positive for \textit{S. aureus}. PRP seemed to inhibit bacterial growth since the amount of effusion decreased after topical application. PRP demonstrated a similar antibacterial function in the second and third cases since neither of the patients was given antibiotics after admission to the hospital.

It was an interesting phenomenon that the first and third patients reported pain relief during the treatment. The same results have been reported by other authors,\textsuperscript{11–13} though the mechanism remains unclear.

**Conclusion**

The application of PRP has been widely used in both experimental and clinical fields. With the advantages of simple preparation, biocompatible safety, low cost, and significant clinical effectiveness, PRP may become an ideal therapy in the treatment of chronic cutaneous wounds. PRP not only releases high concentrations of platelet growth factors to enhance healing,\textsuperscript{9} but also possesses antimicrobial properties that may contribute to the prevention of infections. However, the mechanisms of pain relief, antimicrobial activity, and interactions of multiple growth factors still require further study. Randomized controlled clinical trials are also needed to study the effect of PRP on enhanced restoration of wound structure and function.

**References**