

Use of autologous platelet-rich fibrin on hard-to-heal wounds

This retrospective study found that use of autologous platelet-rich fibrin on a range of hard-to-heal wounds achieved full healing or a significant reduction in wound diameter with no adverse effects. Prospective studies are now needed

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Many factors need to be addressed simultaneously when treating chronic wounds, and healing often does not proceed smoothly. For example, in the case of the diabetic foot, even after infection has been eliminated or avoided, ischaemia and oedema have been treated, and the foot fully offloaded, some wounds still do not heal.¹

Surgical debridement has been performed for decades to restart the wound-healing process; sharp debridement makes the wound bed bleed, and in acute wounds healing starts when a blood clot is formed. Platelets (thrombocytes), which produce a variety of growth factors including platelet-derived growth factor (PDGF) and transforming growth factor-beta, are thought to initiate the healing process.² It is logical to assume, therefore, that the presence of more platelets in the wound bed will aid healing. The number of platelets in blood is approximately $0.2 \times 10^6/\mu\text{l}$. Using purification techniques, the platelet concentration in plasma can be raised to $>1.0 \times 10^6/\mu\text{l}$.³

The use of platelet releasate delivered in a crystalline collagen carrier was first described in 1986.⁴ A subsequent observational study of 49 patients with diabetic foot ulcers receiving platelet-rich material showed that it achieved faster healing times when compared with standard care. The relative risk for a wound to heal after treatment with platelet releasate, compared with standard care, varied from 1.14 (95% confidence interval 1.03–1.27) to 1.59 (1.49–1.70).⁵

Method

Our outpatient wound clinic treats approximately 500–600 patients with standard or complex wounds every year (3000–3500 visits). All types of wounds are treated, but they mostly comprise arterial leg ulcers, diabetic foot ulcers and postoperative wound infections.

Following the successful use of Vivostat platelet-

rich fibrin (PRF) (Vivolution A/S, Denmark) on several patients with hard-to-heal wounds that had failed to respond to standard treatment, we undertook this retrospective study to ascertain whether it promoted healing and caused any adverse effects. As far as we are aware, this is the first study to investigate use of Vivostat PRF on non-healing wounds.

All consecutive patients aged over 18 years with hard-to-heal wounds treated with Vivostat PRF between September 2006 and August 2007 were included in the study. Hard-to-heal wounds were defined as those with granulation tissue that had failed to epithelialise despite two months' of conservative treatment. We did not apply Vivostat PRF to necrotic or sloughy wounds as we thought the platelets would be unable to penetrate the wound bed, or to those with clinical signs of infection. We also considered it unsuitable for patients with anaemia or thrombocytopenia. In all cases, Vivostat PRF was used as a last resort in an attempt to initiate healing. All patients gave informed oral consent to treatment.

Vivolution A/S states that Vivostat PRF can be used in a wide range of surgical procedures and environments where there is a need for growth factors. These include cardiac surgery (healing of the sternum), plastic surgery, orthopaedic surgery (mixing with bone graft) and chronic and surgical wounds.⁶

Using the Vivostat System, practitioners can follow an automated process to prepare the PRF. To do this, 120ml of blood is taken from the patient in accordance with the manufacturer's recommendations and placed in a designated processor unit. In an automated process lasting less than 30 minutes, an average of 6ml of PRF is collected from the 120ml of blood. The PRF is sprayed directly onto the wound, where it polymerises on contact.

We covered the PRF with a low-adherent wound-contact layer (Mepitel, Mölnlycke Health Care) and an absorbent secondary dressing (Vliwasoft, Lohmann & Rauscher). The PRF was left in place for

Table 1. Patient and wound characteristics at the initial presentation and subsequent outcomes following treatment with Vivostat PRF

Patient no.	Sex	Age	Wound type patient	Concomitant treatment	Wound duration (months)	No. of treatments	Outcome
1	Male	59	Diabetic foot ulcer after toe amputation	Offloading (bedrest)	7	1	Closed
2*	Female	75	Mixed aetiology ulcer with calcinosis	Compression therapy	36	1	No difference
3a*	Female	42	Diabetic foot ulcer after forefoot amputation	Offloading (shoes)	2	7	Reduced by 66%
3b			Ulcer following below-knee amputation	Not walking	1	2	Closed
4	Female	67	Radiotherapy ulcer on the breast	–	12	2	Closed
5*	Male	65	Diabetic foot ulcer after metatarsal V amputation	Offloading (wheelchair)	3	4	Reduced by 66%
6	Female	47	Pressure ulcer	Pressure-redistributing mattress	36	4	Reduced by 66%
7	Male	38	Diabetic foot ulcer after toe amputation	Offloading (cast)	8	2	Closed
8	Female	70	Diabetic foot ulcer after (fifth) toe amputation	Offloading (shoes)	24	1	Closed
9	Male	79	Traumatic ulcer	–	3	1	Closed
10*	Female	48	Venous ulcer	Compression therapy	12	3	No difference in diameter but decrease in wound depth
11	Female	89	Ischaemic heel ulcer	–	48	1	Closed
12	Female	47	Traumatic ulcer	–	12	1	Closed

* The three patients still receiving treatment at the time of writing

seven to eight days.⁷ If there were no signs of wound healing, the PRF was reapplied two to four weeks later. All PRF remnants were removed with water and gauze before reapplication. Patients continued with the same modern wound dressing regimen between PRF sessions as had been used before. In addition, they all received standard treatment for their condition, such as compression therapy for venous leg ulceration and offloading for diabetic foot ulcers, in the two months before and during treatment with PRF.

Outcome measures were:

- Full wound closure with no recurrence
- A reduction in wound diameter of up to 66% (initially based on subjective visual assessment and later

Visitrak, Smith & Nephew, which subsequently became available)

- Occurrence of adverse events.

Results

Twelve patients with 13 wounds (one patient had two wounds, which were treated simultaneously) were included in the study. The sample comprised four males and eight females with a mean age of 60.5 years (range 38–89). Most patients were at high risk of anaesthetic/surgical complications: eight were classified as American Society of Anesthesiologists (ASA) class III (severe systemic disease that is not incapacitating) and two as ASA class IV (severe systemic disease that is a constant threat to life).



Fig 1. Patient no. 1: Treatment with offloading, a percutaneous transluminal angioplasty of the crural arteries, TNP and an alginate dressing was unsuccessful



Fig 2. Patient no. 1: After surgical debridement the Vivostat PRF is easily applied using the Spraypen

None showed signs of osteomyelitis or were receiving treatment for arthritis. Patient and wound characteristics are given in Table 1.

The mean wound duration before treatment with Vivostat PRF was 15.7 months (range 1–48). The wound of one month's duration was observed in the patient with two wounds. All wounds were located on the lower limb, with the exception of a radiotherapy ulcer on the breast and a grade IV pressure ulcer (open wound to the bone) on the trunk. The breast wound had been present for one year and was not cancerous.

Seven of the 11 wounds on the lower limb (64%) were associated with chronic ischaemia. In five of the 11 patients, the contralateral leg had been amputated (n=3) or a partial foot amputation performed (n=2).

Other contributors to the wound chronicity in the 12 patients were:

- Obesity (a body mass index of over 25) (n=8, 66%)
- Diabetes mellitus (n=6, 50%)
- Recent history of cigarette smoking (n=2, 17%).

Six wounds were subcutaneous, and the remaining seven had visible muscle or tendon. Unfortunately, we did not document the wound size at the initial presentation, and can only state that eight of the 13 wounds were over 2cm in diameter.

In all cases, the PRF was left in place for the eight days. We would have reapplied the PRF within this period if it had been washed away by high levels of exudate, but this was never necessary.

Eight wounds closed and three wounds reduced in diameter by up to 66%. Two wounds did not reduce in size, although one of these did reduce in depth (Table 1).

The 12 patients received a total of 30 treatments with PRF up to the time of writing (August 2007).



Fig 3. Patient no. 1: The wound is covered with a Mepitel, which is left in place for 5–7 days



Fig 4. Patient no. 1: The closed wound. The patient is fully ambulating, without any problems

Seven of the 13 wounds required more than one application, with a mean number of 2.2 applications. The mean treatment period was 4.2 weeks (range one week to three months).

None of the eight wounds that healed reopened. These wounds were followed up for between one month and 12 months.

No adverse events were reported as a result of the PRF treatment, and none of the patients reported any pain. In all cases, it was possible to complete application (from withdrawal of the blood to bandaging after application of the PRF) within 90 minutes. Where possible, two patients were treated on the same day for logistical reasons. In all cases enough product (mean 6ml) was collected for application.

Three patients were still receiving treatment with Vivostat PRF at the time of writing. One had a venous leg ulcer that had not responded to 12 months' of four-layer bandaging, combined with periods of antibiotic treatment, plus modern dressings including foams and alginates. In this case, PRF was combined with compression therapy, and the wound is now ready to be grafted. The two other patients are being treated with an alginate dressing, and their wounds are getting smaller (progressing from a deep to superficial ulcer).

The first patient we treated, who had diabetes, had undergone a contralateral below-knee amputation and a metatarsal IV and V amputation. Despite offloading, treatment of infection and ischaemia, topical negative pressure and an alginate dressing, his foot ulcer had not healed. The wound, which was over 2cm wide with low exudate levels at the initial presentation, was treated with an alginate dressing, which was changed once or twice weekly. The wound closed after only one application of PRF (Figs 1–4).

Discussion

The literature on the use of PRF on hard-to-heal wounds is limited. A 2006 review of its use on venous leg ulcers called it 'promising'.⁸ Valbonesi reported 'favourable results', based on an unvalidated scoring system, in 11/14 difficult-to-heal acute or

chronic wounds treated with autologous platelets in a fibrin gel.⁹

In Vivostat PRF, autologous fibrin sealant and platelets are combined in an easy-to-use solution that polymerises immediately on application, ensuring the platelet solution stays in place.⁶ It can be applied to wounds on all body sites, including vertical surfaces, and the spray can be inverted. The fibrin releases growth factors, including PDGF, over time, protects endogenous growth factors against proteolytic degradation¹⁰ and increases fibroblast proliferation and subsequent collagen synthesis.¹¹ Growth factors and fibrin/fibrinogen are known to stimulate fibroblast proliferation and migration.¹²

Yazawa et al.⁷ showed that, when incorporated into drug delivery systems such as fibrin, the mean concentration of growth factors in the platelet concentrates was three times or more that observed with conventional platelet-rich plasma. Furthermore, the growth factors were released in a controlled manner over approximately one week.⁷

Finally, a study on the rate of epithelialisation in split-thickness skin-graft donor sites in 20 patients found that those treated with PRF showed 50% epithelialisation after five to eight days compared with 20% for the controls.¹³

Conclusion

The open label nature of this small retrospective study makes it difficult to draw any firm conclusions. However, we believe it shows that treatment with Vivostat PRF in chronic wounds is feasible as there were no problems with application and no complications were reported. Given the chronic nature of the wounds, which had different aetiologies, the high healing rate (62%) is of interest.

However, many questions relating to Vivostat PRF remain, such as its indications and contraindications, application interval and use of secondary dressings. Clearly, further trials are warranted and these should focus on a specific patient group. In our opinion, this should be patients with diabetic foot ulcers, which pose a major clinical challenge to practitioners. ■

References

- 1 Armstrong, D.G., Short, B., Martin, B.R. et al. Maggot therapy in lower extremity hospice wound care. *J Am Podiatr Med Assoc* 2005; 95: 254-257.
- 2 Mercandetti, M., Cohen, A.J. Wound Healing, Healing and Repair. www.emedicine.com/plastic/topic411.htm. Last accessed on 8 January 2008.
- 3 Marx, R.E. Platelet-rich plasma (PRP): what is PRP and what is not PRP? *Implent Dent* 2001; 10: 225-228.
- 4 Knighton, D.R., Ciresi, K.F., Fiegel, V.D. et al. Classification

- and treatment of chronic nonhealing wounds: succesful treatment with autologus platelet-derived wound healing factors (PDWHF). *Ann Surg* 1986; 204: 322-330.
- 5 Margolis, D.J., Kantor, J., Santanna, J. et al. Effectiveness of platelet releasate for the treatment of diabetic neuropatic neuropatic foot ulcers. *Diabetes Care* 2001; 24: 483-488.
- 6 www.vivostat.com
- 7 Yazawa, M., Ogata, H., Nakajima, T. et al. Basic studies on the clinical applications of platelet-rich plasma. *Cell Transplantation*

- 2003; 12: 509-518.
- 8 Anitua, E., Sanchez, M., Nurden, A.T. et al. New insights into an novel applications for platelet-rich fibrin therapies. *Trends in Biotechnology* 2006; 24: 227-234.
- 9 Valbonesi, M., Giannini, G., Migliori, F. et al. The role of autologous fibrin-platelet glue in plastic surgery: a preliminary report. *Int J Artificial Organs* 2002; 25: 334-338.
- 10 Sahni, A., Baker, C.A., Sporn, L. A., Francis, C.W. Fibrinogen and fibrin protect fibroblast growth factor-2 from proteolytic degradation. *Thromb Haemost*

- 2000; 83: 736-741.
- 11 Rybarczyk, B.J., Lawrence, S.O., Simpson-Haidaris, P.J. Matrix-fibrinogen enhances wound closure by increasing both cell proliferation and migration. *Blood* 2003; 102: 4035-4043.
- 12 Hart, J. Inflammation I: its role in the healing of acute wounds. *J Wound Care* 2002; 11: 6, 205-209.
- 13 Danielsen, P., Jorgensen, B., Ågren, M. et al. Effects of topical autologous platelet-rich fibrin (PRF) on epithelialization of human donor site wounds. Abstract book of European Tissue Repair Society meeting, 2006.

The Vivostat System was donated by Vivolution. However, there were no restrictions on the inclusion and exclusion criteria, or relating to publication