



## Clinical Report

# Management of Large Chronic Non-healing Wounds by Autogenous Platelet Rich Plasma and Reconstructive Surgery in Three Cats

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## Abstract

**Case Description-** Three domestic short haired cats (2, 5 and 4 years of age) were presented to the Madras Veterinary College Teaching Hospital, with the history of large chronic, full thickness wounds on the right lateral cervical, left caudal forearm and right lateral thoracic region respectively. Etiology of the wounds was trauma in two cases and thermal burns in the third case.

**Clinical findings-** Physical examination revealed yellowish colored wound with putrid odour and purulent discharge. The subcutaneous tissues were exposed; necrotic tissues and foreign debris were noticed.

**Treatment and Outcome-** In all the three cases, mechanical debridement of the open wound was done by lavaging with sterile normal saline (500 ml) mixed with amikacin sulphate (250 mg) with a 20 ml syringe fixed with a sterile 22 gauge needle to produce a lavaging pressure of 22 psi. Autologous platelet rich plasma was prepared, activated with calcium gluconate(10%) and applied on days 0, 7, 14 respectively on the open wound impregnated with chlorhexidine gauze and bandaging done. Once a healthy granulation bed was formed, reconstructive surgery was planned and performed to close the defect.

**Clinical relevance-** Application of platelet rich plasma remains a viable option for enhancing the granulation tissue formation and early wound bed preparation, before large wounds could be reconstructed with skin flaps. All the above three cases were treated with autologous platelet rich plasma for early granulation and recipient bed preparation followed by reconstructive surgery.

**Keywords-** Cat, Chronic non-healing wound, Platelet Rich Plasma, Reconstruction.

## Case Description

Three domestic short haired cats (2yrs, 5 yrs and 4 yrs of age) were presented with the history of large chronic, non healing wounds on the right lateral cervical (Fig1.A), left caudal forearm (Fig1.B) and right lateral thoracic region (Fig1.C), respectively to the Small Animal Clinic-Out Patient Unit of Madras Veterinary College Teaching Hospital. The etiology of the wounds was trauma in two cases and thermal burn in one case.

The cats were presented two weeks after the injury and were treated by local vet before.

## Clinical findings

Physical examination revealed yellowish colored wound with putrid odour and purulent discharge. The open wounds were on the right lateral cervical, left caudal forearm and right lateral thoracic region respectively. The subcutaneous tissues were exposed; necrotic tissues and foreign debris were noticed. Moderate pain was evinced on palpation of the wound. The cats were in good health and the body temperature, mucous membrane color and capillary refilling time were normal. Blood samples were collected for hematology and serum biochemical analysis and also for preparation of Platelet Rich Plasma (PRP).

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**Figure 1. A:** Case1- Wound on the right lateral cervical region. **B:** Case2- Wound on left caudal forearm. **C:** Case3- Wound on right lateral thorax

### Treatment and Outcome

In all the three cases, mechanical debridement of the open wound was done by lavaging with sterile normal saline- 500 ml (Normal saline, 0.9 % NS, Baxter healthcare, USA) mixed with amikacin sulphate (250 mg, Alfakim, Ranbaxy, India) with a 20 ml syringe fixed with a sterile 22 gauge needle to produce a lavaging pressure of 22 psi.

Autologous platelet rich plasma was prepared by collecting 5 ml of whole blood via jugular venipuncture in K2 EDTA 5.4 mg vacutainer. The first centrifuge was performed at 100g at 4°C for 10 min to separate RBCs

from the remaining whole blood volume in a cold centrifuge. After the first centrifugation step, the whole blood separated into three layers: an upper layer that contained mostly platelets and WBC's, an intermediate layer that is known as the buffy coat and that was rich in WBCs, and a bottom layer that consisted mostly of RBCs. The upper half of the column which contained platelets, buffy coat and some RBC's was transferred to a falcon tube. The second centrifuge was then performed at 400g at 4°C for 10 min. The upper portion of the volume that is composed mostly of PPP (Platelet-Poor Plasma) was removed to create the final volume of 1 ml of PRP (Platelet-Rich Plasma) (Fig 2). The PRP was activated by adding calcium gluconate 10% to the PRP in a 1:2 ratio and gently blended in a sterile Petridish until it reached a gelatinous consistency. The average yield of platelets were  $770 \times 10^3 / \mu\text{L}$ . It was then applied on the open wounds impregnated with Chlorhexidine gauze on days 0, 3, 7 in all the cases and bandaged. Wound planimetry was performed on days 0, 7, 14 by analyzing the captured photos into the "Image J" software which is used to measure the area of the open wounds. Based on this the percentage of epithelialization, wound contraction and healing process was calculated (Table 1). Histological evaluation of the open wound was done on days 7 and 14. After a healthy granulation was formed on the recipient wound bed, transposition flap reconstructive surgery was performed on third week of case presentation in all the cases.



**Figure 2.** Autologous Platelet rich Plasma (PRP)

Pre-operative antibiotics and analgesics were administered. The cats were premedicated with Diazepam-0.2 mg/kg (Lori, Neon laboratories, Mumbai, India) B.W intravenously and Butorphanol tartrate- 0.2 (Butodol-2, Neon laboratories, Mumbai, India) mg/kg B.W intramuscularly. General anaesthesia was induced with Propofol- 4 mg/kg (Neorof, Neon laboratories, Mumbai, India) B.W intravenously and maintained with Isoflurane- 2% (Forane, Abbott pharmaceuticals, India) in oxygen using Boyles anesthetic apparatus. The

surgical areas were prepared aseptically. Flap planning was done meticulously (Fig 3.A and 3.B). The area of the wound was measured in all the cases and flap size was calculated (Table 2). The base of the flap was equal to the width of the defect and the length of the flap was equal to the length from the outer pivot point to the most distant edge of the defect. The donor flaps of the respective cases were marked using a sterile felt tipped marker pen. The flap edges were incised and undermined in a stepwise fashion until the flap could be transposed into the defect with minimal tension. The flap was sutured into position by placing few walking sutures followed by intra dermal sutures with Polyglycolic acid 3-0. Skin was sutured with Polyamide-2-0 in simple interrupted suture pattern. A small corrugated drain tube was placed at the dependent part of the flap and sutured to provide adequate drainage of tissue fluids. The surgical wound was bandaged with an antibiotic ointment every 3 days. Post-operatively, Ceftriaxone- 10 mg/kg (C-tri, Intas Pharmaceuticals, Ahmedabad, India) B.W q12h, Tramadol- 2 mg/kg (Supridol, Neon laboratories, Mumbai, India) B.W q8h and Cetrizine- 0.1 mg/kg (Cetzine, Cipla, Mumbai, India) B.W q8h per-orally was administered for 7 days.

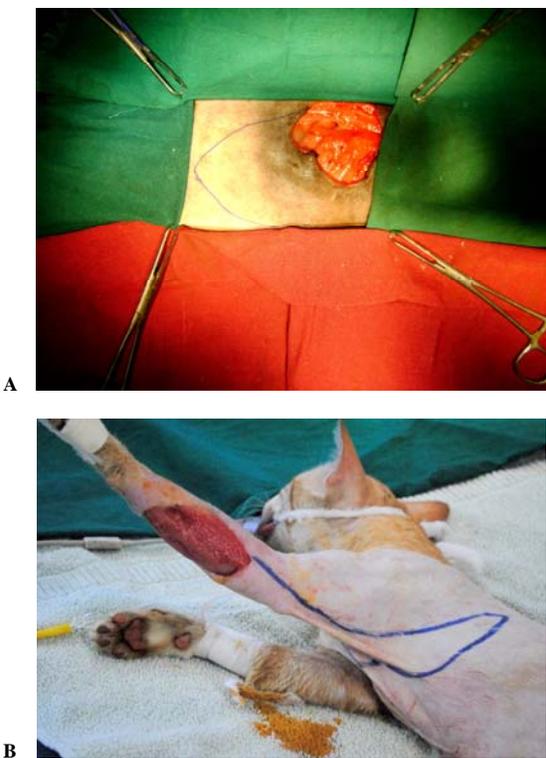


Figure 3. A and B: Flap Planning

Complete flap uptake was observed in right lateral thoracic defect cat and the caudal forearm defect cat. (Fig 4.C and 4.B) The right cervical flap had partial uptake, except for a single 1 square cm region which

was allowed to heal by second intention (Fig 4.A). Sutures were removed on 10th day post operatively. The cats had an uneventful recovery.

A color flow Doppler ultrasound imaging was performed using 7.5 MHz linear probe, on the donor area of skin flap before surgery and also post-operatively on days 3 and 7 to assess the blood flow pattern and perfusion (Fig 5).

Histopathological evaluation of samples taken from the corner of the wound on day 7, and 14 revealed early granulation tissue formation, angiogenesis and epithelialisation when compared to normal wound healing in the control animal, thus describing the efficacy of platelet rich plasma.

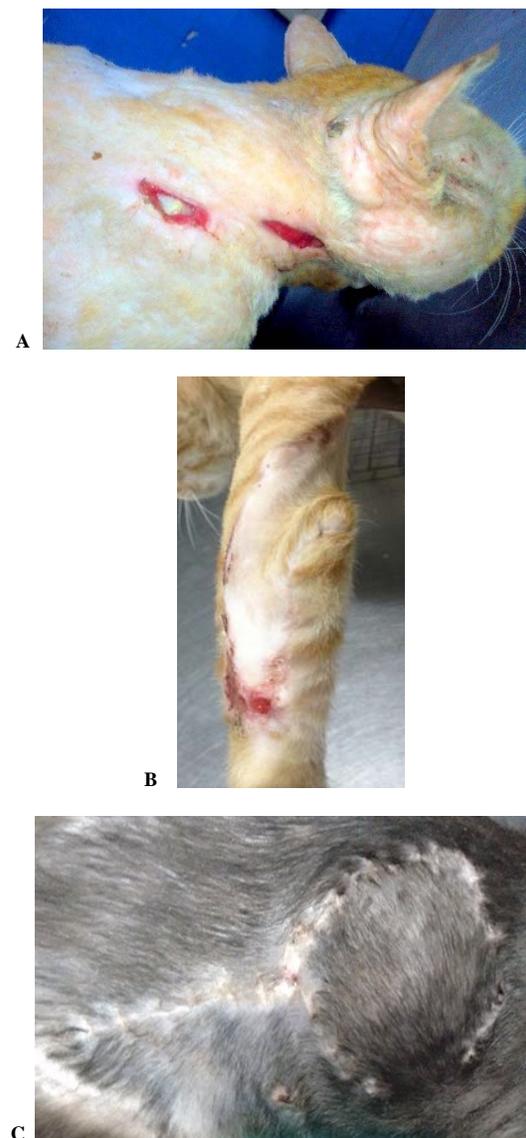
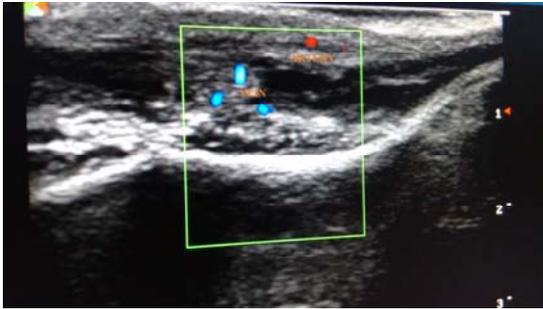


Figure 4. A: Case-1 Flap uptake with mild dehiscence B:Case-2 Complete Flap uptake-Caudal Forearm. C: Case-3 Complete flap uptake-lateral thorax.



**Figure 4.** Vascularity of the flap assessed by Color Doppler Ultrasound, B-mode, 7.5 MHz.

### Clinical Relevance

Skin wounds are commonly seen in veterinary practice. Open wound management, until the wound is considered suitable for reconstruction or until it has healed by second intention, has been the treatment of choice for centuries.<sup>9</sup> Frequent and painful dressing changes over prolonged periods intensifies overall case management, and may impact treatment costs.<sup>2</sup> Moreover, repeated sedation or short anaesthesia is often necessary for dressing changes, discouraging early ambulation and rapid recovery.

Platelet Rich Plasma (PRP) has the supreme advantage when compared to a single growth factor as it offers a multiple synergistically working growth factors that promoted mitogenesis of mesenchymal cells at the wound site.<sup>1</sup> PRP is a concentrate of cytokines and growth factors (GFs) released from platelet alpha-granules at the site of tissue injury.<sup>6</sup> Growth factors like transforming growth factor-B, platelet derived growth factor and vascular endothelial growth factor were released after platelet degranulation<sup>5</sup> which in turn led to fibroblast recruitment, proliferation and matrix remodeling. Ultimately good granulation tissue formation and wound healing was noticed in all the above cases. The increased concentration of growth factors within the autologous PRP would be ideally suited for wound healing in dogs and cat.<sup>3</sup> PRP application on open wounds in cats showed beneficial effects in wound healing by early promotion of granulation tissue, wound contraction and

### References

1. Arguelles D, Carmona JU, Pastor J, Iborra A, Viñals L, Martínez P, Bach E and Prades M. Evaluation of single and double centrifugation tube methods for concentrating equine platelets. *Research in Veterinary Science*, 2006;81: 237-245.
2. Dernel WS. Initial Wound Management. *Veterinary Clinics of North America Small Animal Practice*, 2006;36: 713-738.
3. De Rossi R, Coelho AC, Mello GS, Frazilio FO, Leal CR and Facco GG. Effect of platelet rich plasma gel on the skin healing in surgical wounds in horses. *Acta Cirurgica Brasileira*, 2009; 24: 276-81.
4. Hedlund CS. Large trunk wounds. *Veterinary Clinics of North America Small Animal Practice*, 2006;36: 847-872.

epithelialisation in all the above cases which was evident by wound planimetry histopathological evaluation.

In all the above cases, the number of platelets in the final solution was on average 4.5 times the baseline intravascular platelet count. That is, it was four to five times higher than the baseline count, which is considered the minimal concentration required to accelerate epithelialization and granulation.<sup>7</sup> No systemic or local drugs were used after PRP application. Non-steroidal anti-inflammatory drugs (NSAIDs) were avoided, as they would have inhibitory effect on the treatment.

Transposition flap is a local pedicle flap that transposes skin from an area adjacent to the defect that is to be closed. It is a rotating subdermal plexus flap and it is supplied by terminal branches of the cutaneous arteries and veins.<sup>4</sup> The width of the flap equals the width of the defect. The length of the flap is determined by the pivot point of the flap to the most distant point of the defect. The flap can be rotated into different angles.<sup>8</sup> The most common angle of rotation is 90° and was followed in all the above cases. Trauma causes damage to the cutaneous vessels of the skin around the wound. Hence it is important to assess the integrity of the cutaneous vessels by Doppler ultrasound before planning an axial pattern or a subdermal plexus flap to reconstruct a traumatic wound.<sup>11</sup>

Color Doppler ultrasound technique was relatively easy, non-invasive, and inexpensive method of assessing the integrity of cutaneous arteries and veins.<sup>10</sup> It also helped in assessing the blood flow pattern and perfusion in all the above clinical cases.

PRP is safe, fast, cheap, and simple to produce, and can be used to treat acute and sub-acute wounds in high motion areas like limbs of cats, as well as in cases of delayed wound healing and long-term non healing wounds. There are reports indicating the efficacy of platelet rich plasma in open wounds in dogs and horses. This is the first report of application and clinical use of PRP followed by reconstructive surgery in cats to promote healing in chronic wounds.

5. Iacopetti I, Perazzi A, Ferrari V and Busetto R. Application of Platelet-Rich Gel to Enhance Wound Healing in the Horse: A Case Report. *Equine veterinary science*, 2011; 1-6.
6. Knox RL, Hunt AR, Collins JC, DeSmet M and Barnes S. Platelet-rich plasma combined with skin substitute for chronic wound healing: a case report. *Journal of Extra-Corporeal Technology*, 2006;38: 260-264.
7. Monteiro S, Lepage O and Theoret C. Effect of platelet rich plasma on the repair of wounds on the distal limb in horses. *American Journal of Veterinary Research*, 2009;70: 277-82.
8. Pavletic MM. Skin Flaps in Reconstructive Surgery. *The Veterinary Clinics of North America. Small Animal Practice*, 1990;20(1): 81-103.
9. Pavletic MM. Local flaps. In: Pavletic MM, ed. *Atlas of Small Animal Wound Management and Reconstructive Surgery*, 3 rd ed. Wiley- Blackwell, Ames, IO, 2010; 322-326.
10. Reetz JA, Seiler G, Mayhew PD and Holt DE. Ultrasonographic and colour flow doppler ultrasonographic assessment of direct cutaneous arteries used for axial pattern skin flaps in dogs. *American Veterinary Medical Association*, 2006;228 (9):1361-1365.
11. Stone, CA, Dubbins PA and Morris RJ. Use of colour duplex Doppler imaging in the postoperative assessment of buried free flaps. *Microsurgery*, 2001;21(5): 223-227.

## چکیده

### مدیریت زخم‌های بزرگ مزمن التیام نیافته با استفاده از پلاسمای غنی شده با پلاکت اتولوگوس و جراحی ترمیمی در سه قلاده گربه

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**توصیف بیمار** - سه قلاده گربه نژاد موکوتاه اهلی (۲، ۵ و ۴ ساله) به بیمارستان آموزشی دانشکده دامپزشکی مدارس با تاریخچه زخم‌های بزرگ مزمن که تمام ضخامت پوست را درگیر کرده بود آورده شدند، زخم‌ها به ترتیب در سمت راست گردن، سمت خلفی و چپ بازو و ناحیه جانبی راست توراکس قرار داشتند. علت زخم‌ها در دو مورد تروما و در یک مورد سوختگی بود.  
**علائم بالینی** - معاینه فیزیکی زخم‌های زرد رنگ با بوی تعفن و ترشحات چرکی را نشان داد. بافت‌های زیرجلد قابل رویت بودند، بافت‌های نکروز شده و ذرات خارجی جلب توجه می‌کرد.

**درمان و نتیجه** - در همه موارد، پاکسازی فیزیکی با شستشو بوسیله نرمال سالین استریل (۵۰۰ میلی‌لیتر) مخلوط با آمیکاسین سولفات (۲۵۰ میلی‌گرم) با یک سرنگ ۲۰ سی‌سی و سرسرنج سایز ۲۲ به منظور ایجاد فشار ۲۲ psi هنگام شستشو انجام شد. پلاسمای غنی شده با پلاکت اتولوگوس آماده شد، با گلوکونات کلسیم (۱۰٪) فعال شد و در روزهای صفر، ۷ و ۱۴ روی زخم‌های باز قرار داده شد و بانداز انجام شد. هنگامیکه یک بستر التیامی سالم تشکیل شد، جراحی ترمیمی به منظور بستن نقص انجام شد.

**کاربرد بالینی** - بکار بردن پلاسمای غنی شده با پلاکت به‌عنوان یک گزینه قابل دوام و زیست پذیر برای بهسازی تشکیل بافت التیامی و آماده‌سازی زودهنگام بستر زخم قبل از جراحی ترمیمی با تکه‌ای از پوست مطرح می‌باشد. همه سه قلاده گربه با پلاسمای غنی شده با پلاکت اتولوگوس به منظور ایجاد بافت التیامی اولیه درمان شدند و بعد از آماده شدن بستر مناسب تحت عمل جراحی ترمیمی قرار گرفتند.

**کلمات کلیدی** - گربه، زخم مزمن التیام نیافته، پلاسمای غنی شده با پلاکت، ترمیم.