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# Clinical Application of Autologous Platelet-Rich Plasma (PRP) on Delayed Wound Healing of a Dog with Burns

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**Abstract :** A 9-year-old intact female Poodle (weighing 3.6 kg) was presented for the treatment of a partial and full thickness burn that covering 45% of body including bilateral thigh and sacrolumbar region. Autologous platelet-rich plasma (PRP) stimulates angiogenesis, promoting vascular in-growth and fibroblast proliferation. On the unrecovered right thigh of the dog, autologous PRP was injected into the lesion after application of micro-needles. For macroscopic evaluation digital, photographs were taken from wounds at days 0, 3, 5, 13, 21, and 28. On the 3rd day after PRP application, epithelialization was accelerated. Application of autologous PRP accelerated wound-healing rate and healing time in full thickness burns as well as secondary complications originating from unrecovered wounds. The delayed lesion was completely healed on the 28th day by autologous PRP treatment. In human, PRP was increasingly used in the treatment of a variety of soft tissue in the management of chronic non-healing wounds. This study has shown that PRP treatment can be a valuable and effective aid on intractable wound healing in the dog with burns.

***Key words*** **:** PRP, wound healing, burns, micro-needles.

## Introduction

Burn injury in small animals is commonly associated with electric heating pads, hot packs, ultraviolet/infrared radiation, hot metal, hair drier, boiling liquids (24). The burns vary in recovery depending on the degree and duration of exposure, patient age and healing is usually completed by secondary intention which involves process of epithelialization and contraction in second-deep or more (9,28). However, without proper treatment, it can become nonviable depending on the degree of injury and infection as wound conversion (9).

Micro-needles can be used as pre-treatment tools by making holes in the skin, and semi-solid forms such as ointments, gels or lotions can be used for a local or systemic effect through the hole (16). Micro stimulated skin improve the complex cascade of wound healing, growth factor release, activity of fibroblast and finally results in collagen production (19,22).

Platelet rich plasma (PRP) was initially used for the transfusion in patients with platelets disorder. There are many findings of rich growth factors including PDGF, TGF-β, EGF working on fibroblast collagen synthesis discharged from alpha granules during platelets activation (2,3,29). Autologous PRP that can be applied externally by injection or implant for regeneration is plasma with a higher concentration of platelets increased up to 3~5 times (24). PRP is one of treatment methods in the field of intractable skin diseases, orthopedics, oral surgery, sports medicine and plastic surgery in human beings, and has recently been introduced in orthopedics, dentistry in animal models, and even recently in the veterinary field (1,6,7,13,25,30). Many studies has shown that PRP was effective in chronic plantar fasciopathy, intractable skin disease and skin graft in human (8,13,26). In traditional treatment, there are methods of dressing while taking antibiotics and anti-inflammatory drugs, or honey therapy to treat skin contraction and epithelialization but it is not effective in this case who has delayed recovery in relation to burns, and secondary infection (5,18). Also, there have been few studies on the application of PRP for intractable skin disease in the veterinary study. Therefore, it is needed to study synergy effects of both micro-needle and PRP in the dog.

This is a case study of the effects of autologous PRP and micro-needles in a 9-year-old dog on three-month-old burns that has not recovered and has inflammatory secretions. This case trial has been carried out under the ethical guidelines of Jeju University IACUC.

## Case

Poodle weighing 3.5 kg with body condition score 3 out of 9 was presented for non-appetite, lethargy for 3 days, and large redness skin lesions on the bilateral thigh including thoracic, lumbar region. On the day of admission, the dog was diagnosed with partial and full thickness burns affecting 45% of his body as estimated by calculation of the total body surface area (4,9). The treatment was started with fluid therapy (0.9% normal saline) including enrofloxacin (Baytril®, 5 mg/kg, bid), carprofen (RIMADYL®, Pfizer, Brazil, 2.2 mg/kg, bid), and tramadol (Doranjin®, Samsung pharm, Korea, 4 mg/kg, bid). The lesions were covered with alcohol absorbed

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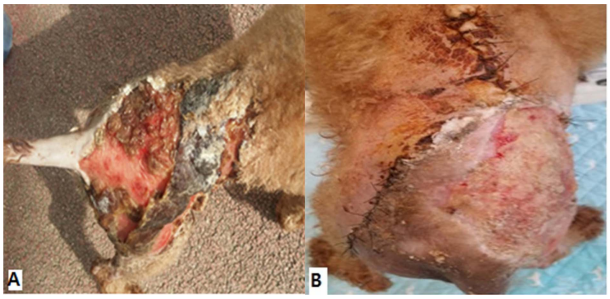

**Fig 1.** Scald burn in a dog. (A) full- thickness burns developed a thick leathery surface of dead tissue, an eschar. (B) full-thickness burns result in complete destruction of all cutaneous structures making surgical intervention necessary.

gauze 3~4 times a day for 3 days. Honey was applied to the wound twice a day in hospitalization.

On the 1st day, along the left lateral thigh, eschar and inflammatory exudates were seen with severe flares. On the 3rd day of hospitalization, eschar and inflammatory exudates were found in the right thigh, thoracic and lumbar spine (Fig 1A). On the 10th day, inflammatory exudates were decreased in the left lesion that had regenerated with healthy granulation tissue. The surgical intervention included debridement and the lesion was washed with warm saline with amikacin (Amikacin®, Samwoo median). Then, suture was performed with intact skin.

The right side of the lesion was epithelialized with surgical intervention and honey therapy for two months (Fig 1B). However, inflammatory exudates crust, and hemorrhage persisted repeatedly in the center of right femoral lesion about 15 $cm^2$ during two weeks. Autologous PRP was added with derma micro needle skin roller 1 mm (DRS540®, Guangzhou EKAI Electronic Technology Co., Ltd) to promote skin healing because client wanted quick recovery. To maximize platelet concentration, whole blood was centrifuged two times (3). Whole blood (15 ml) was drawn by jugular vein, collected, and divided into five EDTA tubes (3 ml K3EDTA, VACUETTE®, Greiner Bio-One., Ltd. Thailand). One of EDTA tubes was used for evaluating on hematological values (Table

**Table 1.** Specific complete blood cell count profile data exacted from platelet rich plasma

| Hematology | Value | | Reference interval |
|---|---|---|---|
| Platelets (K/uL) | 1221 | HIGH | 143-400 K/uL |
| Mean platelet volume (fL) | 9.7 | | 7.0-11.0 fL |
| White blood cell | 0.01 | LOW | 5.20-13.90 K/uL |
| Neutrocyte (k/ul) | 0.06 | LOW | 3.90-8.00 K/uL |
| Lymphocyte (k/ul) | 5.87 | HIGH | 1.00-4.80 K/uL |
| Monocyte (k/ul) | 0.07 | LOW | 0.20-1.10 K/uL |
| Eosinocyte (k/ul) | 0.03 | | 0.00-0.60 K/uL |
| Basocyte (k/ul) | 0.01 | | 0.00-0.10 K/ul |
| Neutrocyte (%) | 1 | LOW | 42.5-77.3% |
| Lymphocyte (%) | 97.1 | HIGH | 11.8-39.6% |
| Monocyte (%) | 1.3 | LOW | 3.3-10.3% |
| Eosinocyte (%) | 0.5 | | 0.0-7.0% |
| Basocyte (%) | 0.1 | | 0.0-1.3% |

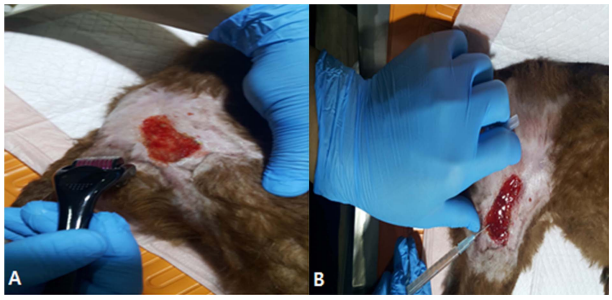

**Fig 2.** Preparation for PRP (A) All area around the lesion were applied with micro-needles 1.0 mm in length more than 3 times. (B) The PRP with 3% calcium chloride, saline was injected into the lesion at several sites.

1, NEODIN co, Ltd). To isolate plasma, tubes were centrifuged for 10 minutes at 230 g (PLC-05, Gemmy Industrial Corp., Taipei, Taiwan), allowing formation of an upper yellowish layer (plasma and platelets), a bottom reddish layer (erythrocytes), and an intermediate whitish layer referred to

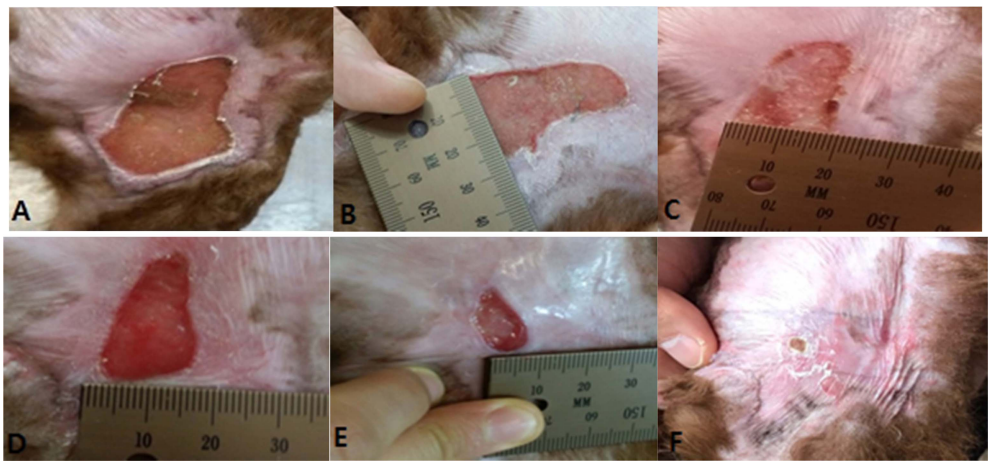

**Fig 3.** Clinical improvement of wound on (A) Day 0, (B) Day 3, (C) Day 5, (D) Day 13, (E) 21 and (F) 28 after PRP injection with micro-needles.

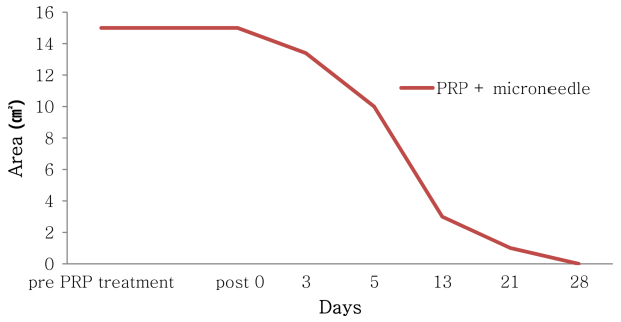


**Fig 4.** Changes in the epithelialization of the burned area after PRP treatment.

as the buffy coat (leukocytes and larger platelets). The entire upper layer and buffy coat were collected into a dry, 5 ml sterile syringe and were centrifuged again for 10 minutes at 2422 g. After the second centrifugation, the platelet-poor plasma (supernatant) was discarded and 0.2 ml of the buffy coat at the bottom of the syringe was transferred into 1 ml syringe. A total volume of 0.8 ml was obtained. Except for saline wash, during treatment, patient was not on any medication, including antibiotics, anti-inflammatory drugs.

The dog had $247 \times 10^3$ platelets/μl when visited in the hospital. Plasma with a platelet concentration of approximately five times greater than the platelet count in normal blood is the working concentration for PRP therapy.

For activating platelet, the PRP (0.8 ml) was mixed with 3% calcium chloride (3% CaCl®, Joongwae pharm, Korea) (0.1 ml) and injected into the lesion through a 1 ml syringe with 21 gauge needle, with several punctures performed (Fig 2).

On the 3rd day after PRP treatment, it induced epithelialization rapidly from the lesion edge and improved in color (Fig 3B). The lesion contracted significantly, with epithelialization and inflammatory secretions disappeared in the 5th day (Fig 3C). The wound significantly healed, almost by half in diameter after the 13th day (Fig 3D). The skin lesion almost completely regenerated with healthy granulation tissue and was healed with epithelialization in 3 weeks after the PRP application with micro-needle (Fig 3E). The wound was completely regenerated after 28 days (Fig 3F).

Compared with 2 weeks in which there was no improvement in the previous treatment, the therapeutic application of PRP with micro-needle was able to confirm the acceleration of epithelialization up to 13 days (Fig 4).

## Discussion

Autologous PRP has been reported in aesthetic medicine related to skin regeneration as well as in sports medicine related to bone, joint, tendon and ligament in human. PRP concentrates a mixture of bioactive agents derived from α-granule of platelets. The α-granule of concentrated platelets activated by aggregation inducers secrets various growth factors including platelet-derived growth factor (PDGF), transforming growth factor (VEGF), and insulin-like growth factor (IGF) (2,10,12,14,15,30).

The use of autologous PRP eye drops could be proven very successful in treating recurrent corneal erosion (17). In grade III burns case, subconjuctival injection of autologous PRP has showed a noticeable reduction in recovery time of conjunctiva (20). In the thoroughbred horse with full-thickness cutaneous wounds under the knee and hock, PRP gel has showed more rapid epithelial differentiation and organization of dermal collagen (7). In human, PRP has increased dermal collagen levels by growth factor as well as skin by skin needling (2). Autologous PRP has showed promise for the treatment of early partial CCL tears in dogs using bone marrow aspirate concentrate or adipose-derived progenitor cells with PRP combination (6).

In small granulating wounds, the topical application of PRP on the distal forelimb of the horse did not improve or accelerate the quality of recovery and the addition of PRP did not obviate the development of exuberant granulation tissue (21). On the contrary, our investigation showed PRP accelerated wound healing massively in untreatable and chronic wounds. In human study, it has showed that patients treated with PRP gel achieved faster healing rates and adequate tissue than conventional treatment group at the end of the second and at the end of the third week (15). Our study resulted to heal more quickly at the first week (Fig 4), and it seemed that micro-needles had played an important role as facilitating efficient delivery. After then, gradual and complete improvement in the dog's skin was noted approximately 1 month after autologous topical PRP application with micro-needles. According to the human split thickness skin graft study compared with the control side by only the applied petrolatum fabric, the defect was markedly epithelialized on the 5th day and was completed in only in the 11th day (14). In our study, results showed an similar potent efficacy for wound healing as that of split thickness skin graft within the 13th day. This showed epithelialization progressed more rapidly.

Previous studies of human platelets in vitro have demonstrated antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, including methicillin-resistant *Staphylococcus aureus* (27). Even without anti-inflammatory, PRP containing hepatocyte growth factor has mechanisms similar to those of non-steroidal anti-inflammatory drugs (30). This study showed PRP application without the use of antibiotics or anti-inflammatory drugs demonstrate those effects as the previous studies.

As a limitation of our study, more biochemical and histological studies are needed to investigate whether PRP can accelerate or improve recovery in wound healing. Further studies with a larger number of samples are required to confirm the skin healing. Considering the increase in platelet count, although PRP was properly separated in our study, this study is based on human methods. This study needs to be applied to lesion site using optimized a centrifugation method for PRP in the dog in the future.

In conclusion, we demonstrated that autologous PRP with micro-needles is non-side effect, efficacious, economical therapy for skin wound healing and may be beneficial to manage chronic, delayed wound therapy in small breed dog.

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