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Comparative study of platelet rich plasma therapy and tilapia fish skin biological bandages for wound healing in cats

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Abstract
The research involved a cohort of twenty-four apparently healthy adult cats divided into two equal groups presented with open wounds, treated with autologous platelet-rich plasma (PRP in group I and tilapia fish skin biological bandaging in group II on days 0, 5 and 10. Assessment of the wound healing revealed increased mean wound contraction percentage over the study period of 21 days in both the groups with no significant difference between the groups. Both groups exhibited robust granulation and epithelialization. In group I, significant reduction in wound area was observed within the group on days 5, 10, 15, and 21, indicating accelerated contraction and faster healing. However, in group II, non-significant reduction in wound area was observed within the group over the same period. Physiological and haemato-biochemical parameters remained stable with no significant difference between the groups on days 0, 8 and 21 of treatment. These findings underscore the almost equal efficacy of PRP and tilapia fish skin biological bandaging in managing open wounds in cats.

Keywords: Cat wounds, felines, platelet rich plasma, regenerative medicine, tilapia fish skin, wound healing, xenografts

Introduction
Wound healing in cats is a crucial physiological process essential for the restoration of tissue integrity following injury. Managing extensive contaminated wounds in cats presents distinctive challenges within veterinary practice, often compounded by the tendency for cats to exhibit prolonged wound healing compared to dogs (Perc and Erjavec, 2022) [17], necessitating specialized attention. While traditional wound dressings have historically been fundamental in wound care, recent advancements in veterinary medicine have introduced alternative strategies.

Regenerative medicine has emerged as a promising avenue to address extensive tissue loss, chronic wounds, and non-healing wounds in veterinary care. One such approach is the utilization of platelet-rich plasma (PRP), an autologous product derived from whole blood through differential centrifugation. PRP releases several platelet-related growth factors that foster activation of resident stem cells, regeneration of endothelial and epithelial tissues, stimulation of angiogenesis, hemostasis, collagen synthesis, and soft-tissue healing. Another such approach of regenerative medicine is the utilization of biological bandages or xenografts for wound healing. These biological bandages mimic normal skin properties, providing better protection against dryness, injury, and infection, thus aiding the healing process. Tilapia fish skin grafts, in particular, offer reduced viral transmission risks and gentle processing that preserves beneficial components like collagen, elastin, and Omega-3 fatty acids, enhancing reepithelialization and microbial defense (Fiakos *et al.*, 2020) [9].

Regenerative medicine aims to focus on growing and replacing damaged tissues and organs, offering longer-lasting and potentially permanent solutions (Mao and Mooney, 2015) [15]. However, to the best of our knowledge, there are a few published reports describing the use of autologous PRP and tilapia fish skin biological bandaging in wound healing in cats. This report seeks to fill this gap by detailing the successful application of autologous PRP and tilapia fish skin biological bandaging on skin defects in cats.

Materials and Methods

The study was conducted on twenty-four apparently healthy adult cats presented with various wounds. The cats were randomly divided into two equal groups, each of 12 animals. The wounds received treatment involving autologous PRP in group I and tilapia fish skin biological bandaging in group II, alongwith antiseptic solutions, ointments, and antibiotics.

For PRP, blood samples were collected aseptically from cephalic or saphenous veins of cats in group I using 3.2% sodium citrate tubes to prevent coagulation. The collected blood was centrifuged in a two-step process as suggested by Theresa (2021) [21] to isolate platelet-rich plasma. Initially, the blood was centrifuged for 5 min at 1431 × g to separate platelets and plasma from other blood components. This step, known as the "soft spin," aimed to isolate the platelet-rich plasma fraction. Following the soft spin, the platelet-rich plasma was carefully transferred to a plain tube. Subsequently, the plasma underwent a second centrifugation step, referred to as the "hard spin," for 10 min at 5724 × g to further concentrate the platelets. Following centrifugation, the resulting PRP was directly injected into the wound bed edges and applied topically as a spray after cleaning and debriding the wound on day 0, 5 and 10. Subsequently, the wound was covered with a sterile dressing to protect against contamination and promote healing.

In group II, tilapia fish skin, used as a biological bandage, was prepared following technique of Choi *et al.* (2021) [5]. The preparation involved scaling, cutting, and cleaning to separate the skin from the body. The skin was rinsed with sterile saline to remove debris, then chemically processed: it was immersed in 2% chlorhexidine solution for 90 min, followed by a 60-min soaking in a 3:1 mixture of 99.5% glycerol anhydrous and 2% chlorhexidine solution. The skin was gently massaged with 99.5% glycerol for 5 min and further sterilized in the same glycerol solution for 60 min. Post-sterilization, the fish skin dressings were placed in open-top transparent plastic packets, exposed to UV light in a vertical laminar airflow cabinet for 5 min, then securely sealed for future use.

Wound size was measured digitally using the Imito Measure smartphone application following guidelines from Bodea *et al.* (2021) [2] on days 0, 5, 10, 15, and 21. Pain level, exudate type, necrotic tissue characteristics, odor intensity, granulation, and epithelialization were assessed on the same days. Percentage wound contraction was calculated using the formula provided by Kodati *et al.* (2011) [13]. The rate of wound healing, defined as the advancement of the wound margin towards the wound center per day was evaluated using the formula from Cukjati *et al.* (2001) [7]. Microbial culture was performed before treatment and after 21 days of application. Overall wound healing was assessed. Heart rate, respiration rate, and rectal temperature were measured on days 0, 8, and 21. Additionally, haematological parameters along with biochemical parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), and creatinine were assessed.

The data was statistically analyzed using completely randomized design one way analysis of variance (ANOVA) as per Snedecor and Cochran (1994) [20].

Results and Discussion

In this study, differential centrifugation method was adopted, which yielded satisfactory results. The mean platelet concentration in whole blood was measured at 3.08±0.13 ($\times 10^5$/mm^3), whereas in PRP, it increased to 24.31±2.10 ($\times 10^5$/mm^3), indicating an 8-fold increase compared to whole blood. According to Saunders *et al.* (2018) [18], a substance qualifies as PRP if its platelet concentration is 2 to 5 times higher than that of whole blood. In this study, the PRP used met this criterion, resulting in successful wound healing.

On days 0, 8, and 21, there were no significant differences observed in heart rate, respiration rate, and rectal temperature, both between and within groups. Furthermore, haematological parameters, including Hb, TEC, TLC, PCV, and platelet count, remained stable with no significant changes. Similarly, biochemical parameters such as AST, ALT, ALP, BUN and creatinine levels did not show any significant alterations throughout the observation period (Table 1).

Table 1: Mean ±SE of physiological and hematobiochemical parameters in cats of treatment group I (PRP application) and II (TFS bandaging)

Parameters	Groups	Day 0	Day 8	Day 21
Mean ± SE (RT) (°F)	Group I	100.69±0.21	100.98±0.17	101±0.23
	Group II	101±0.26	101.03±0.23	100.71±0.27
Mean±SE (HR) (beats/min)	Group I	88.67±3.1	90.67±1.87	93±1.93
	Group II	94.92±1.68	96±1.77	95.25±1.34
Mean±SE (RR) (breaths/min)	Group I	22.58±1.09	20.83±0.89	21.08±0.87
	Group II	22±1.18	21.58±1.20	22.08±1.33
Mean±SE (Hb) (gm%)	Group I	11.26±0.47	10.93±0.33	11.92±0.52
	Group II	11.99±0.5	11.4±0.4	11.73±0.62
Mean±SE (TEC) ($\times 10^6$/mm^3)	Group I	6.93±0.26	7.19±0.87	6.66±0.22
	Group II	6.89±0.14	6.76±0.15	6.79±0.19
Mean±SE (TLC) ($\times 10^3$/mm^3)	Group I	13.48±1.47	11.13±0.56	11.92±0.62
	Group II	14.8±2.05	12.75±0.9	11.97±1.04
Mean±SE (PCV) (%)	Group I	37.46±1.32	36.07±1.31	36.98±1.01
	Group II	37.17±1.59	36.66±1.29	37.81±0.91
Mean±SE (Platelet) ($\times 10^5$/mm^3)	Group I	3.07±0.13	3.09±0.15	3.06±0.95
	Group II	3.07±0.19	3.09±0.12	3.08±0.34
Mean±SE (AST) (IU/L)	Group I	36.33±10.09	36.5±2.97	35.41±3.78
	Group II	54.57±16.47	36.81±3.76	38.86±3.68
Mean±SE (AST) (IU/L)	Group I	42.29±6.67	35.8±2.78	36.63±2.33
	Group II	44.79±7.84	35.87±1.81	35.95±2.06
Mean±SE (ALKP) (IU/L)	Group I	36.33±10.09	36.5±2.97	34.28±3.47

	Group II	29.08±4.58	32.93±3.67	28.47±3.24
Mean±SE (BUN) (mg/dL)	Group I	17.84±0.53	17.33±0.55	19.45±0.91
	Group II	18.88±1.34	17.89±1.03	18.37±1.45
Mean±SE (Creatinine) (mg/dL)	Group I	0.79±0.05	0.78±0.04	0.77±0.04
	Group II	0.8±0.07	0.73±0.04	0.72±0.07

**NS: Non-significant

In group I, the mean wound area (cm^2) measured was 20.1±4.64 on day 0, which subsequently decreased to 16.06±3.83, 13.15±3.29, 9.26±2.38 and 6.19±1.68 on day 5, 10, 15 and 21 respectively (Table 2). This progressive reduction in wound area was found to be statistically significant ($p<0.05$), indicating a beneficial effect of PRP on wound healing. PRP, derived from the patient's own blood, is rich in growth factors and cytokines crucial for tissue repair. In wound healing, PRP stimulates cell proliferation, promotes angiogenesis, and enhances collagen synthesis, essential for the healing process (Smith *et al.*, 2007) [19].

Table 2: Mean ±SE of wound area (cm^2) and wound contraction (%) in cats of treatment group I (PRP application) and II (TFS bandaging)

Parameter	**Groups**	**Days of Treatment**				
		Day 0	**Day 5**	**Day 10**	**Day 15**	**Day 21**
Wound area (cm^2)	I	20.1±4.64^{a}	16.06±3.83^{ab}	13.15±3.29^{abc}	9.26±2.38^{bc}	6.19±1.68^{c}
	II	15.88±3.61	13.32±3.09	10.52±2.65	8.15±2.07	5.22±1.46
Wound contraction (%)	I	0^{e}	20.83±1.23^{d}	37±3.08^{c}	56.41±3.78^{b}	69.67±3.59^{a}
	II	0^{e}	21.17±1.39^{d}	39.67±2.49^{c}	54.42±2.95^{b}	71.83±2.37^{a}

Means with different superscripts within the row differ significantly between time intervals.

In group II, the mean wound area decreased from 15.88 ± 3.61 cm^2 on day 0 to 5.22 ± 1.46 cm^2 by day 21, showing a progressive healing process. However, this reduction was not statistically significant. Group I showed a significant reduction in wound area compared to group II over the same period. There were no significant difference in mean wound area between group I and group II. The prolonged sterilization of Tilapia skin using chlorhexidine might have damaged collagen, compromising the skin matrix integrity (Ibrahim *et al.*, 2020; Erwin *et al.*, 2023) [11, 8]. The mean percentage of wound contraction values on days 5, 10, 15, and 21 were significantly increased within group compared to day 0 ($p<0.01$ for all intervals) in both the group. But, there were no significant differences in wound contraction % between both groups. Notably, wound area decreased significantly with each PRP application on days 5, 10, 15, and 21, but in group II, the decrease was statistically non-significant (Table 2). Epithelialization reached 50-75% in the majority of cases in group I by day 21, accompanied by a significant reduction ($p<0.05$) in wound size on days 5, 10, 15, and 21, indicative of accelerated epithelialization (Fig. 1). These findings were consistent with those reported by Jee *et al.* (2016) [12] and Aminkov (2021) [1] in studies involving dogs. Studies have indicated that PRP can stimulate tissue cells to produce growth factors vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), transforming growth factor-beta (TGF-β), platelet-derived growth factor (PDGF), and insulin-like growth factor-1 (IGF-1), via autocrine or paracrine mechanisms, particularly EGF and TGF-β (Xu *et al.*, 2020) [24]. This mechanism likely contributes to the observed accelerated epithelialization and wound healing in the PRP-treated cases.

Myofibroblasts are the key cells in inducing wound contraction (Xu *et al.*, 2020) [24]. Xu *et al.* (2020) [24] evaluated PRP's impact on myofibroblast formation by examining the expression of the myofibroblast marker α-smooth muscle actin (α-SMA) in wound tissues. They found that PRP enhanced α-SMA production and increased α-SMA positive cells, suggesting PRP's potential to promote myofibroblast formation, facilitate wound contraction, and accelerate healing, as noted in our study.

Group I showed reduction in exudate following PRP applications, aligning with findings by Parmar *et al.* (2022) [16]. In group II, wounds with larger areas had higher exudate levels on day 0, 5, and 10. Excessive exudate led to maceration in five cases by day 5, with minor deterioration in two cases, though not statistically significant. Tilapia skin dressings degraded in these cases, possibly due to over-moisture causing graft detachment and maceration (Erwin *et al*., 2023) [8]. Both the tilapia and PRP groups showed similar trends in reducing wound exudate, but no complications like maceration were observed in the PRP group.

No odor was detected in either group, likely due to consistent wound lavage, dressing, and antibiotics before and during treatment. Additionally, necrotic tissue was absent in all cases on the 0th day of treatment. The reduction in pain was consistent with findings by Carter *et al.* (2011) [4] and Horgos *et al.* (2023) [10] in human studies. Meta-analysis suggested that PRP therapy alleviates pain (Carter *et al.*, 2011) [4] while Horgos *et al.* (2023) [10] noted PRP's role in reducing neurological and neuropathic pain. Pain reduction with tilapia bandages aligned with Costa *et al.* (2019) [6] and Ibrahim *et al.* (2020) [11], highlighting the anti-nociceptive properties of tilapia skin. Pain decreased in group I with each PRP application and in group II with each tilapia skin bandage placement.

Improvement in wound edges was noticeable after each PRP application in nearly all cases. In three complicated wounds with detached edges, reattachment to the wound bed occurred by day 5, indicating treatment effectiveness. In group II, edges exhibited a pink hue and progressive advancement, indicating healing. Both groups had attached and contracting edges without maceration, desiccation, or other abnormalities, except for 2 cases in group II on day 5.

Enhanced granulation was evident in all cases where autologous PRP was utilized. By day 21, the majority of cases achieved 75% to 100% granulation. These findings corroborate with those of Zubin *et al.* (2015) [25], who noted significant granulation tissue formation four days after the initial PRP application. However, our results contradicted those of Parmar *et al.* (2022) [16], who found no significant granulation after PRP treatment in cats compared to dogs. This disparity in findings could be attributed to variations in study design, patient population, or specific treatment protocols employed across different studies. Also, enhanced granulation was observed in group II, with 75%-100%

granulation by day 21, similar to group I. This aligned with Ibrahim *et al.* (2020) [11] and Choi *et al.* (2021) [5], who observed fibroblast proliferation and granulation tissue formation. Results suggested superior granulation with both the treatments.

In group II, epithelialization improved progressively with each dressing change. Both groups I and II exhibited similar epithelialization trends (Fig. 2). Group I showed a statistically significant reduction in wound area whereas, in group II it was non-significant within group. Long-term exposure of Tilapia skin to chlorhexidine during sterilization has been observed to decrease the integrity, organization, and collagen intensity within Tilapia skin. This effect can significantly impact the processes of granulation and neovascularization, as noted by Erwin *et al.* (2023) [8], which is consistent with our findings.

The mean duration of healing was 45.75±5.23 days in group I. Quicker wound contraction was observed on days 5, 10, 15, and 21 when PRP was applied, but delayed healing was observed after the completion of the treatment regimen. The mean rate of wound healing was 0.051±0.003 cm/day. The gradual decrease in growth rate observed after 21 days leads to a delayed or longer duration of healing, ultimately resulting in a slower rate of wound healing. The longer duration of healing might be attributed to larger wound sizes in some cases and instances of self-trauma in a few individuals within the group. Large open wounds in cats often exhibit a slower rate of healing (Bohling *et al*., 2004; Perc and Erjavec, 2022) [3, 17]. This finding aligned with that of Zubin *et al*. (2015) [25] regarding the gradual decrease in growth rate after the completion of the PRP treatment regimen. The mean healing duration for group II was 42.92±3.93 days and the mean wound healing rate was 0.043±0.004 cm/day. This contrasts with Ibrahim *et al*. (2020) [11] and Choi *et al.* (2021) [5], where Choi *et al.* (2021) [5] found tilapia skin grafts promoted epithelialization at 1.76 mm/day, indicating a higher healing rate. No significant difference was found in mean duration of healing and mean wound healing rate between groups I and II.

Localized infection was observed upon case presentation, with microorganisms such as Staphylococcus, Streptococcus and *E. coli* isolated through microbiological examination in both groups. After 21 days, no microorganisms were isolated. Platelet concentrates possess antimicrobial properties, effectively suppressing bacterial growth (Tohidnezhad *et al.*, 2012; Zubin *et al.*, 2015) [22, 25]. Antimicrobial peptides, such as defensins in PRP are essential for host defense, showing bactericidal properties (Vladulescu *et al.*, 2024) [23]. Tilapia fish skin creates a protective shield over the wound site, limiting bacterial colonization (Manzoor *et al.*, 2023) [14]. Both treatments demonstrated effective antibacterial activity, contributing to a healthy wound environment conducive to healing.

PRP treated wound on day 0

PRP treated wound on day 5

PRP treated wound on day 10

PRP treated wound on day 15

PRP treated wound on day 21

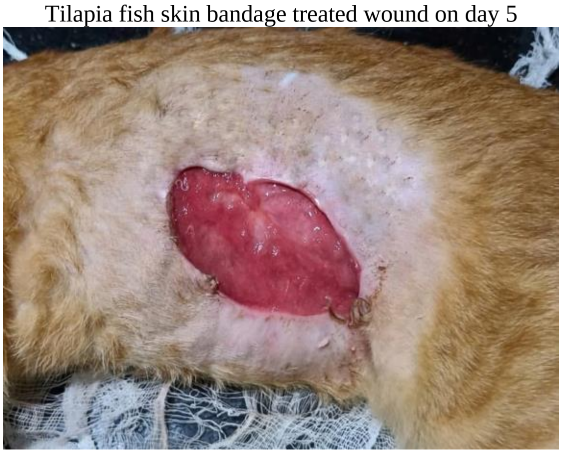
PRP treated wound on day 90

Fig 1: Clinical status of PRP treated skin wound from day 0 to day 90 of treatment in cats

Tilapia fish skin bandage treated wound on day 0

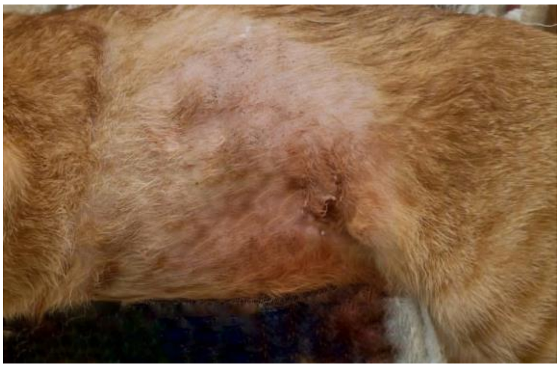
Tilapia fish skin bandage treated wound on day 5

Tilapia fish skin bandage treated wound on day 10



Tilapia fish skin bandage treated wound on day 15

Tilapia fish skin bandage treated wound on day 21



Tilapia fish skin bandage treated wound on day 60

Fig 2: Clinical status of Tilapia fish skin bandage treated skin wound from day 0 to day 60 of treatment in cat

Conclusion

Overall, the wound healing was evaluated as good with both autologous platelet-rich plasma (PRP) topically and intralesionally in group I and tilapia fish skin biological bandaging in group II. By day 21, the mean wound contraction percentage ranged from 60% to 80% in both the groups. Most cases exhibited good granulation (75-100%) and epithelialization (50-75%). There was a gradual decrease in wound area on days 5, 10, 15, and 21 in both the group, but the difference was significant only in group I, indicating quicker wound contraction and demonstrating faster wound healing with PRP. Though the healing rate and duration were similar in both groups, PRP proved to be somewhat more effective than tilapia fish skin bandaging for wound management in cats.

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