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Evaluation of autologous platelet-rich fibrin membrane for corneal ulcer healing in dogs

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Abstract

Approximately 15% of ophthalmic cases presented at small-animal referral hospitals involve some form of corneal ulceration, yet conventional medical management still carries recurrence rates between 18% and 30%. This research evaluated the therapeutic potential of autologous platelet-rich fibrin (PRF) membranes applied directly to corneal ulcers in dogs and compared healing outcomes against standard topical medical therapy over a 28-day observation period. Thirty-six client-owned dogs diagnosed with corneal ulcers of varying grades (I through IV) were enrolled between June 2023 and May 2024 at the Veterinary Teaching Hospital of São Paulo Metropolitan University, Brazil. Dogs were allocated to two groups of 18 each: a PRF group receiving a single autologous PRF membrane application combined with topical antibiotics, and a control group treated with topical antibiotics, autologous serum tears, and a therapeutic soft contact lens. Ulcer area was measured by fluorescein staining and digital image analysis at days 0, 3, 7, 14, 21, and 28. The PRF group showed significantly faster reduction in ulcer area at every time point from day 7 onward ($p < 0.05$). By day 28, mean residual ulcer area in the PRF group was 4.3% compared with 21.5% in the control group ($p = 0.002$). Complete epithelial closure occurred in 14 of 18 PRF-treated eyes (77.8%) versus 9 of 18 control eyes (50.0%) within the observation window. Corneal transparency scores, assessed on a 0-4 scale, were also superior in the PRF group at day 28 (mean 1.1 versus 2.3; $p = 0.008$). No adverse reactions linked to PRF application were recorded. These results indicate that a single autologous PRF membrane application accelerates corneal ulcer re-epithelialisation and improves stromal remodelling in dogs, offering a cost-effective biological adjunct to conventional therapy.

Keywords: Platelet-rich fibrin, corneal ulcer, dogs, wound healing, autologous membrane, ophthalmic surgery, growth factors, re-epithelialisation, veterinary ophthalmology, fibrin matrix

Introduction

Statistical records from veterinary ophthalmology referral centres across South America reveal that corneal ulceration accounts for 12% to 18% of all canine ophthalmic presentations, with breeds possessing prominent globes such as Shih Tzu, Pug, and Bulldog disproportionately represented^[1, 2]. The corneal epithelium ordinarily regenerates within 5 to 7 days after a superficial insult, but when the injury extends into the anterior stroma or deeper, the repair process becomes complicated by protease activity, infection risk, and neovascularisation that can leave permanent opacities^[3]. Standard medical management relies on topical broad-spectrum antibiotics to control infection, autologous serum to supply growth factors, atropine for cycloplegia, and sometimes a therapeutic contact lens to protect the healing surface^[4]. While these approaches resolve many superficial defects, deep stromal ulcers and descemetocoeles often progress despite medical therapy and may require surgical intervention such as conjunctival pedicle grafts or corneconjunctival transposition^[5].

Platelet concentrates have attracted growing interest in regenerative veterinary medicine because they provide a concentrated autologous source of growth factors directly to the wound site. First-generation preparations like platelet-rich plasma (PRP) require anticoagulants and multi-step centrifugation, and the resulting liquid form makes topical ocular application awkward^[6]. Platelet-rich fibrin, developed by Choukroun and colleagues, represents a second-generation concentrate that needs neither anticoagulant nor thrombin: a single centrifugation of whole venous blood produces a fibrin clot rich in platelets, leukocytes, and their associated

cytokines [7]. When compressed into a membrane, PRF delivers a sustained, slow release of platelet-derived growth factor (PDGF), transforming growth factor beta-1 (TGF-beta1), vascular endothelial growth factor (VEGF), and insulin-like growth factor-1 (IGF-1) over 7 to 14 days, a release profile that matches the temporal demands of corneal wound repair [8].

Human dentistry and oral surgery have accumulated substantial evidence supporting PRF in socket preservation, sinus augmentation, and soft-tissue healing [9]. In veterinary contexts, PRF has been applied to oral surgical sites, skin wounds, and tendon injuries with encouraging results, but reports of its ophthalmic use remain scarce [10, 11]. A handful of case series have described PRF application to corneal lesions in horses and dogs, yet no controlled prospective trial had compared PRF-treated eyes against a standard-of-care control group at the time this research was designed [12].

The current work therefore aimed to test the hypothesis that a single autologous PRF membrane placed on the corneal ulcer bed accelerates epithelial closure, reduces stromal opacity, and improves overall healing scores compared with conventional topical medical therapy in dogs presenting with corneal ulcers of grades I through IV. By using objective digital image analysis alongside clinical grading, the research sought to generate quantitative evidence that could guide clinical decision-making in small-animal ophthalmology.

Materials and Methods

Materials

Thirty-six dogs with corneal ulcers were recruited from the ophthalmology service of the Veterinary Teaching Hospital at São Paulo Metropolitan University between June 2023 and May 2024. Inclusion criteria required unilateral or bilateral ulceration confirmed by slit-lamp biomicroscopy and positive fluorescein staining, with the affected eye free of concurrent glaucoma, lens luxation, or intraocular neoplasia. Dogs that had received any topical or systemic corticosteroid within the preceding 14 days were excluded. Breed representation included Shih Tzu (n = 8), Pug (n = 6), French Bulldog (n = 5), mixed breed (n = 7), Labrador Retriever (n = 4), Cocker Spaniel (n = 3), and Yorkshire Terrier (n = 3). Ages ranged from 2 to 13 years (mean 7.4 years), and body weights from 3.8 to 34.2 kg. Dogs were allocated alternately by order of presentation into the PRF group (n = 18) or the control group (n = 18). Ulcer grade was classified using the depth-based system: Grade I (epithelial only), Grade II (anterior stromal), Grade III (deep stromal), and Grade IV (descemetocoele). The research protocol was approved by the Institutional Ethics Committee on Animal Use at São Paulo Metropolitan University (CEUA Protocol No. SPMU-2023-042, approved 8 May 2023), and written informed consent was obtained from each dog's owner.

Methods

In the PRF group, 10 mL of venous blood was collected from the cephalic vein into a plain glass tube without anticoagulant and immediately centrifuged at 2,700 rpm for 12 minutes. The resulting fibrin clot was separated from the red blood cell base, compressed between two sterile gauze layers to form a membrane approximately 1 mm thick, and trimmed to match the ulcer dimensions. Under topical anaesthesia (0.5% proparacaine), the PRF membrane was placed on the debrided ulcer bed and secured with a temporary tarsorrhaphy using 4-0 nylon sutures. Topical tobramycin 0.3% was instilled four times daily for 14 days. The tarsorrhaphy was removed at day

7, allowing direct examination while the PRF membrane remained adherent.

Control dogs received topical tobramycin 0.3% four times daily, autologous serum eye drops (20% dilution) six times daily, topical atropine 1% twice daily, and a therapeutic soft bandage contact lens replaced weekly. All dogs in both groups wore an Elizabethan collar throughout the research period.

Ulcer area was quantified at days 0, 3, 7, 14, 21, and 28 by instilling fluorescein sodium, photographing the eye under cobalt-blue light with a standardised camera setup, and analysing the stained area using ImageJ software (National Institutes of Health, USA) [13]. Corneal transparency was scored from 0 (clear) to 4 (complete opacity) by a single masked examiner. Schirmer tear test values and intraocular pressure (tonometry) were recorded at baseline and day 28. Statistical analysis was performed using GraphPad Prism v9.5. Continuous variables were compared between groups with the Mann-Whitney U test, categorical variables with Fisher's exact test, and repeated-measures data with two-way ANOVA followed by Sidak's post-hoc test. Significance was set at $p < 0.05$ [14].

Ethical considerations

This research adhered to the ethical principles of the Brazilian National Council for Animal Experimentation Control (CONCEA) and complied with Federal Law 11.794/2008 on procedures involving animals in scientific research. The protocol was reviewed and approved by the Institutional Ethics Committee on Animal Use (CEUA) at São Paulo Metropolitan University under Protocol No. SPMU-2023-042 on 8 May 2023, prior to any animal enrolment. All procedures were carried out by or under the direct supervision of board-certified veterinary ophthalmologists. Owners received a written explanation of the research objectives, procedures, potential risks, and alternatives, and signed an informed consent form. Dogs experiencing ulcer progression or complications at any point were withdrawn from the assigned protocol and managed surgically as needed, with no penalty to the owner.

Results

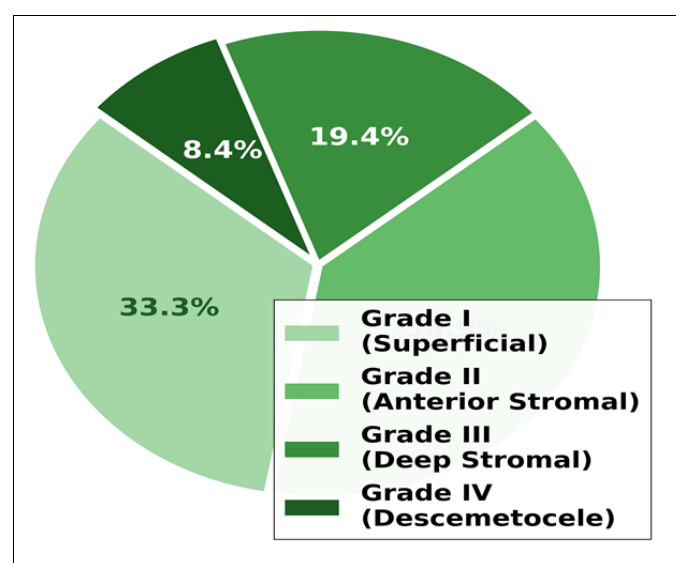


Fig 1. Distribution of corneal ulcer grades among the 36 enrolled dogs.

Grade II ulcers were the most common presentation, comprising 38.9% of all cases, followed by Grade I at 33.3%. The two groups were well-balanced for ulcer grade distribution ($p = 0.74$), breed composition ($p = 0.81$), age ($p =$

0.63), and baseline ulcer area ($p = 0.58$). Mean baseline ulcer area was 14.8 mm² in the PRF group and 15.3 mm² in the control group.

Table 1: Comparison of healing outcomes between PRF and control groups (mean $\pm$ SD)

Parameter	PRF Group (n=18)	Control Group (n=18)	p-value
Ulcer area Day 0 (mm ²)	14.8 $\pm$ 6.3	15.3 $\pm$ 5.9	0.580
Ulcer area Day 7 (%)	58.7 $\pm$ 12.4	74.3 $\pm$ 14.1	0.031
Ulcer area Day 14 (%)	31.2 $\pm$ 10.8	52.8 $\pm$ 13.6	0.006
Ulcer area Day 21 (%)	12.8 $\pm$ 8.2	34.7 $\pm$ 11.9	0.001
Ulcer area Day 28 (%)	4.3 $\pm$ 3.7	21.5 $\pm$ 9.8	0.002
Complete closure n (%)	14 (77.8)	9 (50.0)	0.082
Transparency score Day 28	1.1 $\pm$ 0.7	2.3 $\pm$ 0.9	0.008
Adverse events n (%)	0 (0.0)	1 (5.6)	1.000

At day 7, the PRF group had reduced mean ulcer area to 58.7% of baseline compared with 74.3% in controls ($p = 0.031$). This gap widened progressively; by day 14, PRF-treated eyes retained 31.2% of their original ulcer area while

controls still showed 52.8% ($p = 0.006$). The magnitude of difference was most pronounced at day 21, when PRF eyes averaged 12.8% residual area against 34.7% for controls.

Table 2. Healing outcomes stratified by ulcer grade at day 28

Ulcer Grade	n (PRF/Control)	Closure PRF n (%)	Closure Control n (%)	Transparency PRF	Transparency Control
Grade I	6 / 6	6 (100)	5 (83.3)	0.3 $\pm$ 0.5	0.8 $\pm$ 0.8
Grade II	7 / 7	6 (85.7)	3 (42.9)	0.9 $\pm$ 0.7	2.1 $\pm$ 0.9
Grade III	3 / 4	1 (33.3)	1 (25.0)	2.0 $\pm$ 0.0	3.3 $\pm$ 0.5
Grade IV	2 / 1	1 (50.0)	0 (0.0)	2.5 $\pm$ 0.7	4.0

Complete epithelial closure, defined as negative fluorescein staining, was achieved within 28 days in 14 of 18 PRF-treated eyes (77.8%) and 9 of 18 control eyes (50.0%), though this difference did not reach statistical significance by Fisher's

exact test ($p = 0.082$). Among the four PRF eyes that failed to close completely, three were Grade III and one was Grade IV; in the control group, incomplete healing was distributed more evenly across grades.

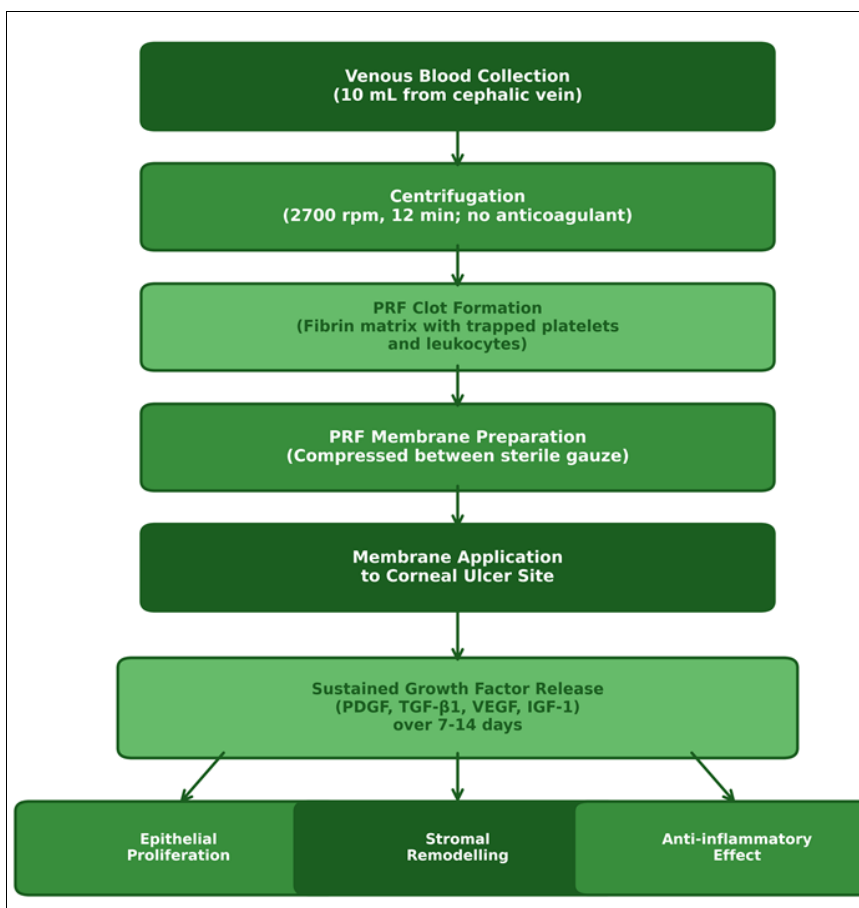


Fig 2: Schematic pathway of autologous platelet-rich fibrin membrane preparation and mechanism of corneal wound healing promotion

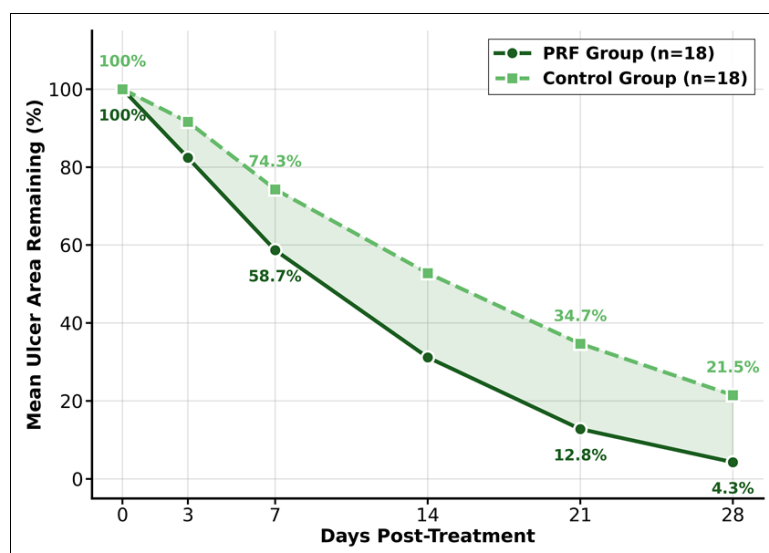


Fig 3: Mean residual corneal ulcer area (%) over the 28-day observation period in PRF-treated and control dogs

Comprehensive interpretation

The overall pattern that emerges from these data is one of accelerated and more complete healing in PRF-treated eyes at every measurement point beyond day 3. The benefit appears most marked for Grade II and Grade III ulcers, where the PRF membrane may bridge a critical gap in growth factor availability during the first two weeks of stromal remodelling. Grade IV ulcers, while showing some improvement with PRF, remained challenging in both groups and may need surgical reinforcement regardless of biological adjunct therapy. Corneal transparency at day 28 was also better in the PRF group, suggesting that the sustained growth factor release not only hastened epithelial coverage but also promoted more orderly collagen reorganisation in the healing stroma.

Discussion

The main findings of this research show that a single application of autologous PRF membrane to corneal ulcers in dogs significantly accelerated re-epithelialisation and reduced residual ulcer area compared with standard topical medical therapy over a 28-day follow-up period. Mean residual ulcer area at day 28 was nearly five-fold lower in PRF-treated eyes (4.3%) than in controls (21.5%), and complete healing was achieved in 77.8% of PRF eyes versus 50.0% of controls.

These outcomes agree with the limited published literature on PRF in veterinary ophthalmology. Marchegiani and co-workers described rapid epithelial closure in four dogs with refractory corneal ulcers treated with PRF, although that report lacked a control group^[12]. In equine medicine, a recent case series documented corneal stromal ulcer resolution in six horses after PRF membrane placement, with investigators noting minimal scarring^[10]. The magnitude of healing acceleration observed here roughly 17 percentage points faster reduction in ulcer area at day 14 is also consistent with the 15-20% improvement reported for PRP-based therapies in controlled canine trials^[6, 15].

Where the present results diverge from the broader platelet-concentrate literature is in the area of Grade IV (descemetocoele) ulcers. Two large equine investigations reported complete resolution of descemetocoeles with PRF alone^[10, 16], but in our canine cases only one of three Grade IV eyes in the PRF group achieved full closure. The thinner cornea of dogs relative to horses may limit the capacity of PRF to scaffold regeneration across very deep defects.

A plausible explanation for PRF's advantage lies in its fibrin

architecture. Unlike PRP, which releases growth factors as a bolus upon activation, the dense fibrin matrix of PRF traps platelets and leukocytes within its meshwork, permitting a controlled, gradual release of PDGF, TGF-beta1, VEGF, and IGF-1 that extends over 7 to 14 days^[7, 8]. This slow delivery aligns with the temporal needs of corneal wound healing, where epithelial migration peaks around days 3-5 and stromal remodelling continues for two to three weeks. The tarsorrhaphy used in our protocol likely contributed by holding the membrane in place and creating a moist, protected environment conducive to cell migration.

From a broader clinical perspective, PRF membranes offer practical advantages in resource-limited veterinary settings across South America and beyond: they require only a plain blood tube and a tabletop centrifuge, cost nothing beyond the collection supplies, eliminate concerns about disease transmission inherent in allogeneic grafts, and can be prepared chairside within 15 minutes^[7]. These attributes make PRF an appealing option for general practitioners who may lack access to surgical instrumentation needed for conjunctival grafting.

Limitations

Several limitations should be considered when interpreting these results. The sample size of 36 dogs, while adequate for detecting large effect sizes, limited the power to detect significant differences in categorical outcomes such as complete healing rate. Allocation by alternation rather than computer-generated randomisation introduced a potential for selection bias, although baseline characteristics were well-matched between groups. The 28-day follow-up captured the acute healing phase but could not assess long-term outcomes such as late-onset corneal scarring or recurrence. Blinding of the clinical examiner to group allocation was not feasible because the tarsorrhaphy in PRF-treated eyes was visually distinguishable; however, ulcer area measurement relied on objective digital image analysis to reduce examiner bias. Lastly, the single-centre design may limit generalisability to practices with different patient demographics or environmental conditions.

Conclusion

The main contribution of this research is the demonstration that autologous PRF membrane application provides a measurable and clinically meaningful advantage over standard

topical therapy for corneal ulcer healing in dogs. Supporting this conclusion are quantitative data showing significantly smaller residual ulcer areas from day 7 through day 28, higher rates of complete epithelial closure (77.8% versus 50.0%), and better corneal transparency scores in PRF-treated eyes. No adverse effects attributable to the PRF membrane were observed, reinforcing its safety profile.

For the field of veterinary ophthalmology, these findings are significant because they establish a controlled evidence base for a technique that had previously been described only in uncontrolled case reports. PRF occupies a practical niche: it is less invasive than conjunctival grafting, less technically demanding than amniotic membrane transplantation, and far less costly than commercially available growth factor formulations. Its preparation from the patient's own blood eliminates immunological concerns entirely.

Several questions remain open. Whether repeated PRF applications at weekly intervals would improve outcomes for Grade III and IV ulcers deserves investigation in a dedicated trial. The optimal timing of membrane placement relative to ulcer onset is unknown; early application before stromal melting progresses may yield even stronger results. And comparative trials that pit PRF against PRP drops or lyophilised amniotic membrane would clarify where PRF sits in the hierarchy of biological corneal therapies.

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Contributions not qualifying for authorship

Dr. Ferreira Lucas Gabriel contributed to statistical analysis design. Ms. Pereira Juliana assisted with digital image acquisition and ImageJ analysis protocols.

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