Effects of Autologous Platelet-Rich Fibrin (PRF) Therapy on Wound Healing in Dogs

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Research Article

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Abstract

Background: Platelet-rich fibrin (PRF), also recognized as leukocyte-rich-PRF, is a recent platelet-based biomaterial, pointed as an innovative regenerative strategy for the treatment of wounds, from different etiologies. This study aimed to evaluate the efficacy of autologous PRF as a regenerative therapy for the treatment of skin wounds of multiple etiology, in dogs. The authors postulated that autologous PRFs could be successfully used as a biocompatible patch to increase the physiologic wound regeneration, acting as a biological antiseptic biomaterial, in spontaneous open large or chronic wounds in dogs.

Eight dogs, aged between 7-months to 9-year-old, with naturally occurring cutaneous wounds were enrolled in this study. Four of these wounds were infected. The PRF treatment was initially performed two times per week, followed by single weekly treatments from the second week onwards, until exophytic granulation tissue was present. The study was finalized when complete wound closure was achieved.

Results: PRF grafting treatments were well tolerated in all treated wounds, inducing significant granulation tissue formation. PRF clots acted as a natural tissue-filler, promoting the epithelization and wound closure, without the requirement of topical antimicrobial/antiseptics application, or additional debridement. Evident skin contraction was recorded in larger injuries and all the treatments resulted in vestigial aesthetic scars.

Conclusions: The PRF therapy constitutes a promising regenerative treatment, accelerating the natural healing process.

Background

Platelet-rich fibrin (PRF), also recognized as leukocyte-rich-PRF (1), is a recent platelet-based biomaterial, pointed as an innovative regenerative strategy for the treatment of wounds, from different etiologies (2–8).

Recent research supports the therapeutic use of PRFs, defined as a biocompatible and biodegradable natural scaffold, constituted by a fibrin matrix, containing elevated amounts of platelets and leukocytes, having the capability to release high concentrations of bioactive structural proteins, along time (9). The PRFs contain abundant concentrations of platelets and leucocytes, above the physiologic baseline, considered essential elements for wound regeneration, to stimulate local angiogenesis, cellular migration, proliferation, and differentiation (10).

Studies focused on the temporal profile of the PRFs' secretome have demonstrated that the bioactive proteins are released from the clots, being maintained at the wound bedding even after its application (9, 11). Several growth factors (GFs), such as platelet-derived growth factor-BB (PDGF-BB), transforming growth factor β-1 (TGF-β1), and vascular endothelial growth factor-A (VEGF-A), as well other important cytokines, are released after platelet and/or leukocytes degranulation, initiated during the centrifugation process (12, 13). A recent *in vitro* study from our group, demonstrated the active release of PDGF-BB, TGF-β1 and VEGF-A from canine PRFs until day 10 after its production, and verified an initial burst release of interleukin-8 after one day of PRF preparation (14).

The PRF success in human clinical context has been supported by both *in vitro* and *in vivo* scientific research (9, 15–17). The increasing interest in platelet-based therapies has also contributed to the development of novel veterinary treatments (18, 19).

This research reports the clinical performance of autologous PRFs as biocompatible patchs, potentiating the physiologic wound regeneration in naturally occurring skin wounds in domestic dogs. PRFs acted also as a biological antiseptic biomaterial, in spontaneous medium to large sized open wounds.

Results

Macroscopic analysis of the wounds

All PRF treatments were well tolerated (Fig. 1).

Each PRF-grafting procedure, in all the patients, induced a noteworthy granulation tissue formation, a highly friable and intense-reddish material at the wound bedding. In most cases, the mainstream PRF clots applied were not visible in subsequent treatments, although they could be found in particular instances attached and integrated within the granulation tissue (Fig. 2).

In case 3, the epithelization process was more evident than granulation tissue formation after the PRF-therapy. This wound was considered closed at 28 days, despite being considered having deficient epithelization. Nevertheless, 3 weeks after it was open, and a digit amputation was performed as a squamous cell carcinoma lesion was diagnosed in the excised tissue.

The molecular analysis of the animal in case 4 revealed the infection by *Leishmania* sp. This patient did not complete the study, and a humanized euthanasia was performed, as requested by the owners. Nevertheless, the wound of this canine received two PRF-treatments, and in 2 weeks, the wound was reduced in 42.92%.

Four of the assessed wounds were infected at day 1, containing purulent exudate. Case 1, resulted from a dog bite injury, and according to the owner, it would have around six hours. Nevertheless, the characteristics revealed by the medical examination of the injury, namely the presence of purulent yellow material with odor, and the inflammatory satellite dermatitis in the surrounding tissue (Fig. 1, case 1/ day 1) suggested that the injury would have happened longer ago.

The presence of exudate was observed, especially in the larger and deeper wounds, at the initial time points of the grafting-treatments. Nevertheless, no infection signs were observed in the peripheric area and/or in the wound bedding during the PRF treatments. Ischemic or necrotic tissue was not observed in any of the cases, under any circumstance. The complete wound closure (n = 7) occurred in a medium period of 31 days (13–42 days), following an estimated reduction rate of 0.23 cm^2/ day.

Wound contraction, re-epithelialization, and crust formation were progressively perceived, in all the cases. Most of the cases resulted in dry crust formations, except for case 7, where a serous crust was observed after day 18, breaching down with sterile saline solution cleansing (Fig. 3-a).

At the scheduled time point (day 24) the owner reported that the dog was rubbing the affected ear, and an external otitis in the affected ear caused by *Malassezia* sp. was cytologically diagnosed. Additionally, the interior facet of the pinna was also injured. At this time and considering the absence of a severely exudative ear canal, the affected ear channel was cleaned with a lipolytic and keratolytic veterinary solution, and the dog started topical auricular treatment.

All the wounds recorded a notorious centrifugal wound healing, from the periphery to the center of the lesion. Moreover, all the treatments resulted in vestigial aesthetic scars. Evident skin contraction was recorded in the larger skin injuries (Fig. 3-b). In the seven cases that concluded the present study, 2 wounds relapsed after PRF therapy ended (Fig. 1, cases 2 and 3). The other dogs recorded no wound recurrence for a follow-up period of 2 up to 12 months.

Assessment of the wound area along time

The wound area of each skin wound documented throughout time is characterized in Table 1 (detailed description in a table format, Additional file 1).

Table 1
Resumed characterization of the wounds treated by PRF-therapy, treatment protocol and events documented in the study (considering D1 as the baseline).

Case	Initial wound area (cm^2)	Wound healing duration	Number of PRF-treatments/ Total number of wound treatments	Total number of PRF clots applied	Score of cleanliness/ contamination degree of wounds (21)	Ongoing Therapeutic drugs	Adverse effects and follow-up period
1	6.15	13 days	3/4	10	4	ATB NSAID	Not observed After 2 months: no wound recurrence. Skin with normal appearance.
2	12.02	42 days	5/7	17	4	ATB NSAID ANALG	Not observed. A nodule (± 1cm) was identified dorsally to the lesion, during the wound healing process. After 10 months: wound recurrence (small area), associated with 2.3 cm diameter. Owners accepted the surgical removal of the digit (histopathology revealed an extraskeletal chondrosarcoma).
3	0.75	28 days	3/5	2	3	ATB (started to be administrated 1 week before PRF therapy)	Not observed After 3 weeks: wound recurrence. Surgical amputation of the digit was performed, and histopathology revelled a dermic squamous cell carcinoma, not detected by appositional cytology performed at the initial consult presentation.

Case	Initial wound area (cm²)	Wound healing duration	Number of PRF-treatments/ Total number of wound treatments	Total number of PRF clots applied	Score of cleanliness/ contamination degree of wounds (21)	Ongoing Therapeutic drugs	Adverse effects and follow-up period
4	4.95	15 Days	2/3	4	4	ATB NSAID	Not observed during the treatments. This animal did not complete the study, being euthanized after the confirmation of *Leishmania* sp. Infection.
5	6.70	32 days	5/8	12	4	ATB NSAID ANALG	Not observed. After 6 months: no wound recurrence. Skin with normal appearance.
6	11.21	31 days	5/7	10	3	ATB NSAID ANALG	Not observed After 12 months: no wound recurrence. Skin with normal appearance.
7	6.29	31 days	4/7	7	3	ATB NSAID TOP-ANTIFUNG (> D24) Not observed	Not observed After 12 months: no wound recurrence. Skin with normal appearance.
8	0.41	23 days	2/6	2	2	Not administrated After D14: ointment containing vitamin A + zinc applied only in the interdigital plantar region	Not observed After 2 months: no wound recurrence. Skin with normal appearance.

The wound area recorded for each lesion is expressed in Fig. 4-a. In all wounds, a consistent and significand contraction of the skin were observed within the initial 2 weeks of PRF therapy, revealed by a slope in the wound contraction percentage curve plot, for each treated wound (Fig. 4-b).

The initial surface area of the eight treated wounds ranged from 0.41 cm^2 to 12.02 cm^2, with a median surface area of 6.22 cm^2 (IQR 1.80 to 6.22 cm^2), at day 1, immediately before the first PRF treatment.

A progressive median wound contraction percentage along the PRF-therapy was calculated in the 7 cases enrolled in the study: 30.88% (IQR 25.675 to 49.11%) at the end of the first week of treatment (until day 7), increasing to 62.76% (IQR 47.48–84.18%) at the second week (until days 14–15), with one lesion achieving the complete closure (case 1). Also, at this time point, case 4 did not proceed any further (as previously stated, Fig. 1, case 4/ day 15).

At the third week (from 15 to 21 days), six cases were ongoing, recording a median wound contraction of 79.41% (IQR 65.22–81.63%).

At the fourth week of treatment (days 22–28), from the six cases being assessed, two achieved the complete healing at this period (Fig. 1, case 3/ day 28 and case 8/ day 23), with a median wound contraction of 94.74% (IQR 82.93–100.00%).

At the fifth week of treatment (days 29–35), three of the four ongoing cases concluded the study (Fig. 1, case 5/ day 32, case 6/ day 31 and case 7/ day 31). The only wound still not completely healed in the study, at this time, was the larger lesion which achieved complete resolution at day 42 (week 6, Fig. 1, case 2/ day 42).

A statistically significant positive correlation was found between the initial wound area and the wound healing duration ($rs = 0.8289$, $p = 0.0278$). There was also a statistically significant positive correlation between the initial wound area and the number of PRF treatments performed ($rs = 0.9543$, $p = 0.0048$). However, no correlation was found between the duration of the wound healing process and the number of PRF clots applied at the first treatment (day 1), or the total number of PRF clots applied during the complete treatment ($rs = 0.3056$, $p = 0.5000$ and $rs = 0,7000$, $p = 0.0857$, respectively). A statistically significant correlation was found between the total number of PRF clots applied and the score of cleanliness and condition of the wounds ($rs = 0.8758$, $p = 0.0214$).

Discussion

It is currently accepted that platelets play a determining role in wound regeneration (22). Platelet-rich fibrin rational use stems from the fact that the clot acts as a natural tissue filler, being an important trigger for the local healing progress, promoting both neoangiogenesis and tissue remodeling, by releasing high concentrations of bioactive structural proteins (9). Nevertheless, platelets are not the single cellular elements with central role in the healing process. The concentration of leukocytes observed within the PRF also directly guarantees the tissue remodeling and regeneration (23).

The PRF use in canine patients has been rarely described. Studies have demonstrated the efficacy of PRF in dogs for tissue healing and regeneration of post-extraction sites in animals with spontaneous periodontal disease, reducing the expression of inflammatory cytokines such as TNF-α and IL-1β, and stimulating the expression of collagen production-associated genes such as COL1A1, COL3A1, and TIMP1, and of the GFs – PDGF-B, TGF−β1, and VEGF-A (24, 25). Additionally, PRF has gain interest in human context, being considered a hemoderivative with regenerative potential. There are several advantages accredited to this autologous biomaterial, being its preparation high reproducibility one of them, with low donor-to-donor variability in its composition (19, 26–28).

The GFs released are recognized as endogenous peptides that regulate both fibroblast and peripheral stem cell migration, proliferation, and differentiation, also promoting angiogenesis, which is crucial for wound healing processes (2, 29, 30). Also, progenitor stem cells were detected in platelet concentrates, the majority from the hematopoietic lineage (CD34+/CD45+), having the capacity to promote the maturation of endothelial cells and local neoangiogenesis; stem cells from nonhematopoietic lineages (CD34+/CD45-) were also found, having the ability to differentiate into mesenchymal cells (e.g. osteoblasts, chondrocytes) (31).

Data collected within this study demonstrated a significant difference in wound progress, confirmed by the reducing of all injury areas over the time, suggesting a sustained healing pattern provided by a local delivery of high concentration of both GFs and cytokines, released from the PRF matrix (19, 32).

Granulation tissue is an important component in the skin injury repair process, comprising new thin-walled capillaries, which fill the wound, during the healing by second intention (33). This tissue has been described as a contractile material, characterized by the proliferation of fibroblasts, endothelial cells and keratinocytes, colonized by local inflammatory cell population, in a complex cellular and molecular crosstalk within the wound region (34). In the present study, each PRF-grafting procedure was initially performed two times per week in order to increase the *in situ* concentration of bioactive peptides at the wound site. Overall, single PRF treatments were applied from the second week onward, until the acquisition of a notably proliferative and exophytic granulation tissue. Closed bandage with an imbued vaseline gauze was then applied, until epithelization and wound resolution was reached. The cases presenting extended or profound injuries (1, 6, 7 and 8), and one case with moistening crusted lesions due to its localization (case 8, lesion in the interdigital location) required additional regenerative biomaterial in the second week. In all the cases, PRF clots were perfectly integrated into the wound site, being locally degraded, with no rejection or necrosis.

A progressive wound contraction was documented in this study, more evident within the initial two weeks of PRF-therapy: the second PRF-grafting procedure was executed 4.5 days (median) after the initial wound presentation, associated with a wound contraction of 30.88%, at the first week. A median wound contraction of 62.76% was achieved between days 8 and 15 after two PRF-grafting treatments (n = 8), where case 1 accomplished the complete wound closure. A statistically positive correlation was found between the initial wound area, the wound healing duration, and the number of PRF treatments performed. Therefore, larger wounds require a higher number of PRFs, and longer time to heal. Nevertheless, the duration of the wound healing process is not associated with the number of PRF clots applied at the first treatment (day 1), or with the total number of PRF clots applied during the complete treatment. Contaminated wounds or wounds with higher scores required higher number of PRF clots applied.

Two of the seven PRF-assisted cases that achieved wound closure, experienced wound relapse. In these cases, histopathological examination exposed the presence of neoplastic lesions (an extraskeletal chondrosarcoma in case 2, and a dermic squamous cell carcinoma in case 3), that were deemed to be the relapsing cause. In case 2, previous imprint cytologic exam was negative for neoplastic cells. Recent works have studied platelet-derived formulations, such as platelet-rich plasma (PRP), as a co-adjuvant therapy in cancer treatment, helping the slower growth of the tumor (35, 36). In the past, the role of GFs on tissue angiogenesis and tumor progression has been suggested, but there is insufficient data regarding this matter. The authors strongly recommend biopsy examination on recurrent or chronic lesions before start PRF therapy.

Nonetheless, the influence of PRF treatments on the development of the neoplasia has never been clearly stated by human clinical researchers using PRF therapy, and it is important to note that being a recent methodology, there are still many unexplored features (23, 37, 38).

PRF-therapy has claimed to have effective topical antimicrobial activity (39–41).The inexistence of wound infection along this research may uncover an important intrinsic antimicrobial property of PRF clots, especially considering that no local antiseptic was applied and that four wounds included in this study were unequivocally infected. This study found a statistically significant correlation between the score of cleanliness/ contamination of wounds and the total number of PRFs applied. Case 8 was treated only with PRF therapy (no systemic antibiotic or anti-inflammatory), and case 3 had antibiotic administration only in the first 3 days of PRF-therapy. Considering case 7, a topical ear treatment was administered due to an external otitis in the last third of the treatment (> day 24). The otitis may have delayed the wound healing process in this animal, as the owner reported that the dog was trying to scratch the affected ear.

Once that all the cases were referred from a general veterinary clinician already receiving systemic antimicrobials, the suspension of these therapy was not recommended with the integration of the animals in PRF treatments, due to the associated risk of a possible antimicrobial resistance drawback. The bacterial culture of the wounds was not performed once that antimicrobials were already being administrated, and the first PRF treatments contributed to a significant clinical improvement of the lesions. Nevertheless, the authors would like to state that a bacterial culture test with a respective sensitivity analysis is recommended when treating skin wounds with significative tissue loss, complicated, or chronic, in the alignment of current veterinary medical practices. Nevertheless, in all cases the antimicrobials administrated were aligned by current veterinary clinical guidelines, considering that: four wounds were clinically infected and with purulent discharge or exudate (cases 1, 2, 4 and 5); two cases have passed for surgical interventions immediately before PRF therapy (cases 5 and 6); two cases had open wounds with tissue loss (cases 3 and 7), being these animals living outdoor, contacting with soil frequently, and therefore, these wounds were assumed to be contaminated. Nonetheless, the clinical outcomes attained by PRF therapy support that this technique may reduce the use of additional antimicrobial/chemical agents generally required for wound management, adding to the ecological and biodegradability properties of this biomaterial.

Regarding the technical features of PRFs' manufacturing, using fresh blood without anticoagulants, when the treated wound required more than one PRF and 5 mL syringes for the collection of each corresponding PRF, was crucial to obtain consistent PRF clots. The blood harvest should be constant without excessive pressure (that occurs when small volume syringes are used), inhibiting vacuum over the vein and its collapse.

The authors recognize the absence of a control group as a limitation of this study. However, the authors considered that having a group with no implemented treatment would be unethical. Nevertheless, a control group consisting in wounds treated with physiologic saline solution for comparison with wounds treated with PRF-therapy would not be representative of the healing process evolution, since the wounds documented in this study occurred spontaneously, and therefore, with different etiologies. Furthermore, the clinical experience of the research group allows to infer those treatments with PRFs are faster than conventional treatments with commercial ointments, and easier to handle during wound healing.

Beyond its efficiency performance, PRF-methodology revealed to be an easy, cost-effective therapy, with a simple obtention protocol, and with low variability in clot formation between individuals. Furthermore, PRF-therapy technique can be pointed as an environmental protective medical technique: polypropylene tubes used to produce the standardized PRFs were reused, envisioning the waste reduction, and supporting ecological practices in clinical research. The tubes were used after both appropriate decontamination and washing, followed by autoclave sterilization (14, 42). Polypropylene is a plastic polymer that is cost-efficiently produced, thermoresistant, robust, with antifouling properties, with low cell attachment, free from absorption of hydrophobic drug molecules and water evaporation (43). The reduced use of antibiotics is also a major feature of this technique, addressing one of our biggest issues in clinical approaches under the *One Health* context, the antibiotic resistance.

The results from this study sustain PRF therapy as an innovative biomedical methodology for the regeneration of skin lesions, from different etiologies, where second intention healing is required, leading to the stimulation of progressive wound regeneration.

Conclusions

The results of this study demonstrated that autologous PRF clots constitute an efficient regenerative wound therapy. The PRF-grafting technique can be used to treat medium to large sized wounds, or even chronic wounds (small or large), granting the formation of healthy augmentation tissues in injuries with tissue loss, and even in infected lesions. Owing to its stimulatory effect on angiogenesis and epithelialization, PRF is an excellent biomaterial, acting as a temporal-release patch, locally discharging bioactive peptides that endorse the physiologic restoring of tissue integrity. Additionally, PRF application may reduce the use of additional antimicrobial/chemical agents generally used in wound management, unveiling a possible antimicrobial local effect, and an environmental protective medical technique. Furthermore, PRF-therapy constitutes a biological cost-effective strategy, reducing the need for surgical reconstructive interventions, occasionally restricted by the existence of possible patient’s morbidities, by possible clinical post-operative complications, or even by economical restraints.

Methods

The clinical proposal

This study aimed to evaluate the therapeutic efficacy of autologous PRF grafting as an *in situ* hemoderivative grafting biomaterial for the treatment of naturally occurring skin wounds, characterized by tissue loss, in domestic dogs.

Canine population and production of autologous PRF clots

This study was approved by the Ethics Committee of the University of Trás-os-Montes-e-Alto-Douro (Doc03-CE-UTAD-2020) and has the writing consent of the animals' owners.

Eight domestic dogs (5 females and 3 males), aged between 7-months to 9-year-old, most of them presenting medium to large critical size skin lesions, but also small chronic skin lesions, were enrolled in this study (Table 2). The animals were firstly assisted by a general veterinary clinician, being after referred to a veterinary clinician specialized in regenerative wound therapies. The animals presented lesions from different etiologies, such as ulcerations, bite wounds, lacerations, tissue-loss, surgical sutures dehiscence, some of them with necrotic tissue. Four wounds were considered infected (cases 1, 2, 4 and 5).

Table 2
Demographic and clinical characterization of the dogs enrolled in the study.

Case	Age	Sex	Weight (kg)	Breed	Origin of the lesion and localization	Characteristics of the lesion	Habitat regime of the animal
1	4 ye	Female, intact	13	Cocker Spaniel	Dog bite, Left cervical region	Infected wound (< 6 hours)	Mixed (in and outdoor)
2	8 ye	Female, spayed	40	German Shepherd	Subcutaneous ruptured abscess, Right scapular region	Necrotic tissue Infected wound (> 1 day) [A]	Outdoor
3	7 ye	Male, neutered	32	Retriever Labrador	Traumatic wound, Paw pad hind limb	Chronic wound (> 1 month, sutured previously, but recurred)	Outdoor
4	9 ye	Male, intact	30	Crossbreed German Shepherd	Ulcerative lesion due to Leishmaniosis (confirmed 1 week after), Right anterior carpus	Infected wound (1 week)	Outdoor
5	5 ye	Female, spayed	7	Crossbreed	Dehiscence of surgical repair of a dog-bite lesion, Left cervical region	Infected wound (3–4 days) [B]	Mostly indoor
6	9 ye	Male, neutered	28	American Pit Bull Terrier	Surgical wound dehiscence due to a radical surgical tumor resection, Left metacarpus	Accentuated tissue loss and ligament exposition (2 days)	Mixed (in and outdoor)
7	8 ye	Female, spayed	38	Crossbreed Portuguese Serra-da-Estrela	Myiasis (maggot larvae of flies) infestation, Right posterior ear pinna	Accentuated tissue loss Cartilage exposition (1 week)	Outdoor

Case	Age	Sex	Weight (kg)	Breed	Origin of the lesion and localization	Characteristics of the lesion	Habitat regime of the animal
8	7 mo	Female, intact	13	Border Collie	Laceration in the interdigital region, II-III digits, Right anterior paw	Chronic wound (with 24 days), suffering dehiscence after 2 suturing procedures, and 1 surgical tissue adhesive application	Mixed (in and outdoor)

A. Wound was detected the day before, although the abscess was present before.

B. The traumatic wound resulted from a dog-bite attack, and was previously surgically repaired in another clinic, with the application of surgical drains, before being admitted and incorporated in this study.

Legend: mo, months-old; ye, year-old.

Protocol for the production of the PRFs

Each PRF clot was produced using an aseptic technique as previous described (14, 18, 20). Briefly, peripheric venous blood was collected to sterilized conical base polypropylene tubes (57 x 15.3 mm), without clot activator. Five milliliters of whole blood were drawn to produce one PRF-clot. When the treated wound required more than 1 PRF, a 19 G butterfly catheter and 5 mL syringes were used for multiple blood collections. The centrifugation at 580 *g* (3000 rpm) was immediately performed after the blood collection, for 10 minutes, at room temperature. It was used an in-clinics 45º angle rotor centrifuge (Orto Alresa® RT 114, NS 080214/02, ø 8.2 cm). The blood was left to rest inside the tube for a maximum time of 60 minutes after the centrifugation step, for the cases in which PRF polymerization did not occur during or immediately after the centrifugation step. The PRF clots were harvested from the tubes, the red fraction was removed using sterile materials, and the PRF was ready to be grafted (Fig. 5).

Considering the emerging global environmental plastic-related issues, the polypropylene tubes used in this research were reused, after its decontamination, followed by manual cleaning and autoclave sterilization.

PRF protocol: grafting procedure and treatment

At the first day of treatment (D1), all the wounds were scored from 1 to 4 according to its cleanliness and condition score (1: clean wound; 2: clean-contaminated wound; 3: contaminated wound, with no necrosis, or necrotic tissue without infection: 4: dirty-infected wound, necrosis and pus present, traumatic wounds greater than 4 hours old) (21). Thereafter, the skin wounds were mechanically debrided, and necrotic tissue, if present, was removed. Thereafter, before each new treatment, all the lesions were irrigated with sterile saline solution to remove all inflammatory exudate or debris (Fig. 6).

The number of PRFs applied was depended on each lesion size. The PRF clots were grafted accordingly to the following statements:

1. each PRF treatment was performed by applying newly produced PRFs at the recipient area, assuring the contact between the PRFs platelet and leukocyte-rich area, and the lesion;
2. one PRF was applied for each 1.5–2 cm^2 of wound area, and a maximum of 4 PRFs/ wound were applied (maximal capacity to produce 4 PRFs/ treatment);

3. in the absence of a sufficient number of clots to fill the entire wound defect and if skin detachment of the wound borders was observed, the periphery of the wounds was privileged for PRF-grafting (since the granulation tissue is created in a centripetal form);
4. in the absence of detachment of the wound borders, the PRFs were preferentially grafted in the area of main tissue deficit (where the granulation tissue was firstly required, and considering that the granulation tissue growths from deeper areas towards the surface);
5. after PRF application, a closed bandage was applied using a sterile vaseline gauze bandage, maintaining the clot at the recipient area.

Most of the patients received conventional systemic pharmacological, implemented by the first assistant veterinary clinician (stated in a table format, Additional file 2).

Each PRF treatment was initially performed two times per week. Single treatments were applied from the second week until wound epithelization was reached, in most cases, according to the owner’s schedule. The application of PRFs was suspended once proliferative and exophytic granulation tissue was observed in the wound bedding. Continuity of the bandaging procedure was performed equally, until complete wound closure was reached.

Wound area evaluation and statistical analysis

The wound area was documented and assessed at each time point. The wound depth was not considered for evaluation. Most of the patients were also re-evaluated at the minimum 2 months after wound closure.

The wound area during the healing progress was calculated by the same researcher using ImageJ® software (version Image J: 2.1.0/1.53c, Bethesda, MD, USA). A Spearman's rank correlation coefficient (rs) was applied to determine the association between the initial wound area, the wound healing duration, number of PRF treatments performed, the number of PRFs applied at the first treatment (day 1), total number of PRFs applied and the wound contamination score. The percentage of wound contraction (%WC) was calculated using the following formula: [(Wound Area at day 1 – Wound Area at Specific Time point) / Wound Area at day 1] × 100 (20). Results were expressed as the median, and interquartile ranges (IQR). Statistical analysis was conducted using Prism® (Version 6 GraphPad® Software Inc., La Jolla, USA). Statistically significance was considered as $p < 0.05$.

Declarations

Ethical approval and consent to participate

This study was approved by the Ethics Committee of the University of Trás-os-Montes-E-Alto-Douro (Doc03-CE-UTAD-2020). All the methods were carried out in accordance with relevant guidelines and regulations. Furthermore, all methods are reported in accordance with ARRIVE guidelines for the reporting of animal experiments. The owners provided informed consent, authorizing the integration of the animals in this study.

Consent for publication

Not applicable in the declarations section.

Availability of data and materials

The dataset(s) supporting the conclusions of this article are included within the article (and its additional file(s).

Conflict of interest

Pedro P. Carvalho is the C.E.O. and Founder of Vetherapy, a biotechnology company that commercializes cellular therapies for veterinary medicine. All authors declare that there is no conflict of interest regarding the publication of this paper.

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Author Contributions

C.S., I.D., M.A.P. and P.C. contributed to the study design; C.S. contributed for sample and data collection; C.S., I.D., M.A.P. and P.C. contributed for data analyses and drafting of the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

Macroscopic documentation of the treated wounds, according to the respective time point assessment. Wounds from the cases 1,2,4, and 5 were infected, presenting purulent exudate.

Legend: D, day; END, finalization of the study/ complete wound closure; *, last presential appointment, and crust detachment was reported by the respective owners few days later (2-4 days).

Figure 2

Appearance of the wound in case 5, after three autologous PRF grafting procedures. In this case, the clot previously applied was still visible (yellow arrowhead) in this treatment, integrated in the new-formerly granulation tissue.

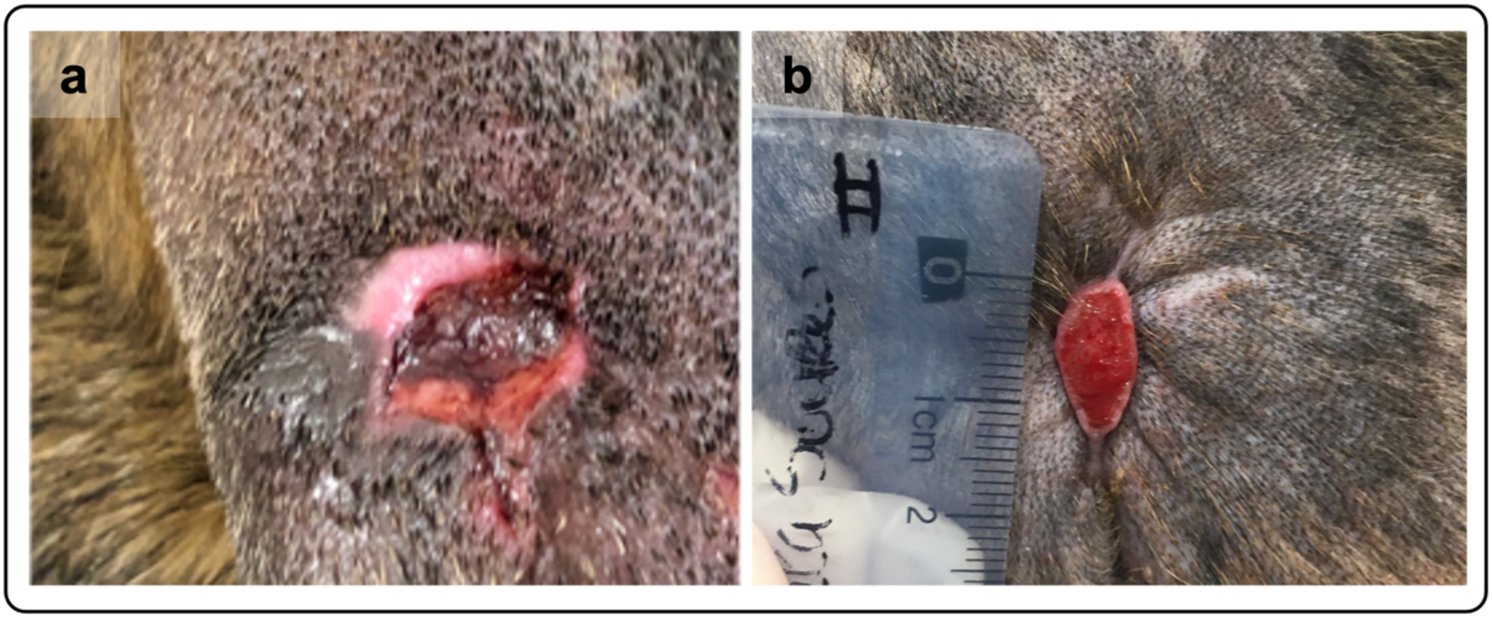

Figure 3

Features of wound closure documented along the study: (a) serous crust at day 24 and mild wound contraction (case 7); (b) considerable wound contraction observed at day 26, with epithelization formation (case 5).

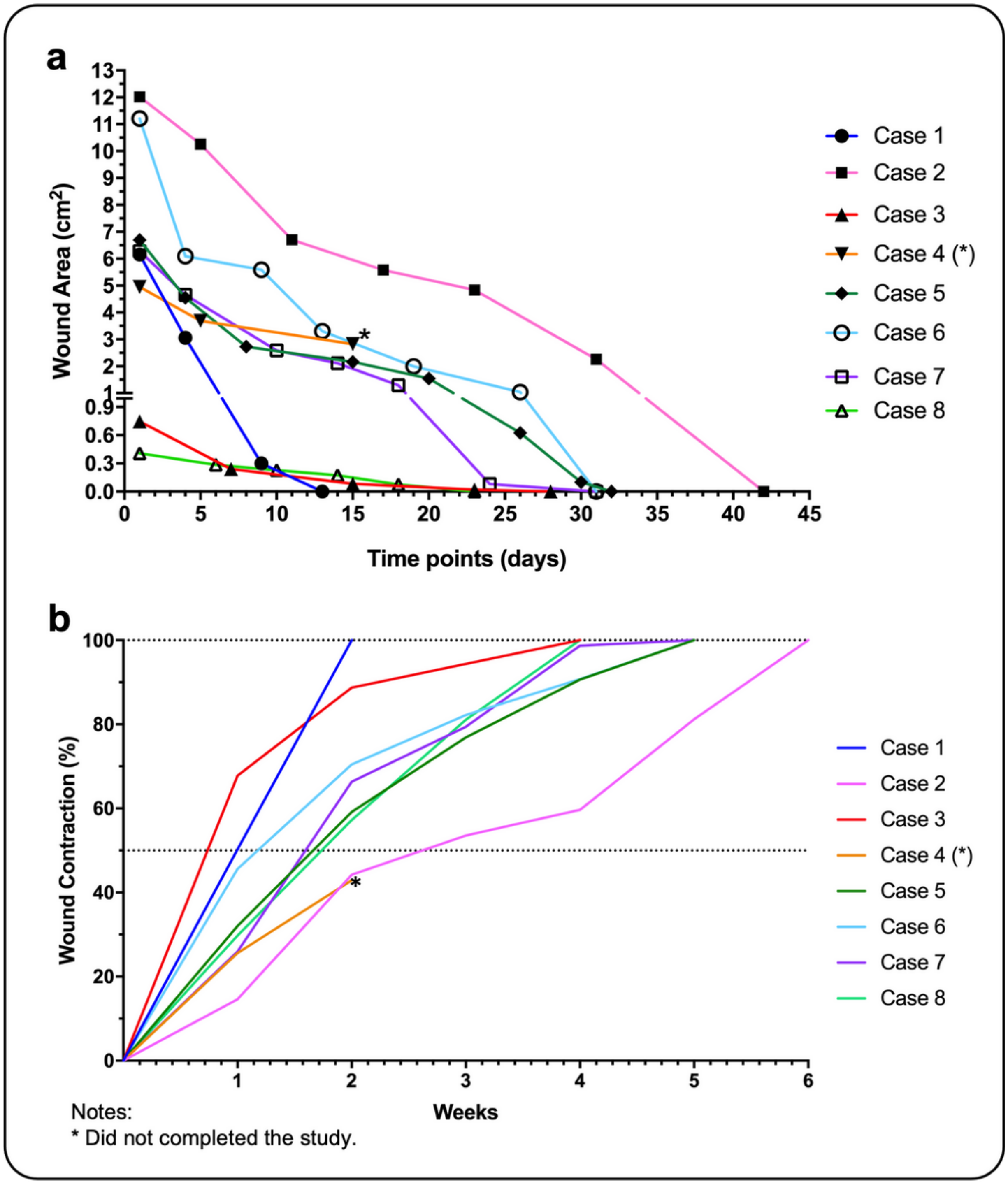

Figure 4

Quantitative assessment of wound area (a) and respective percentage of contraction (b) documented for each case and time point assessment.

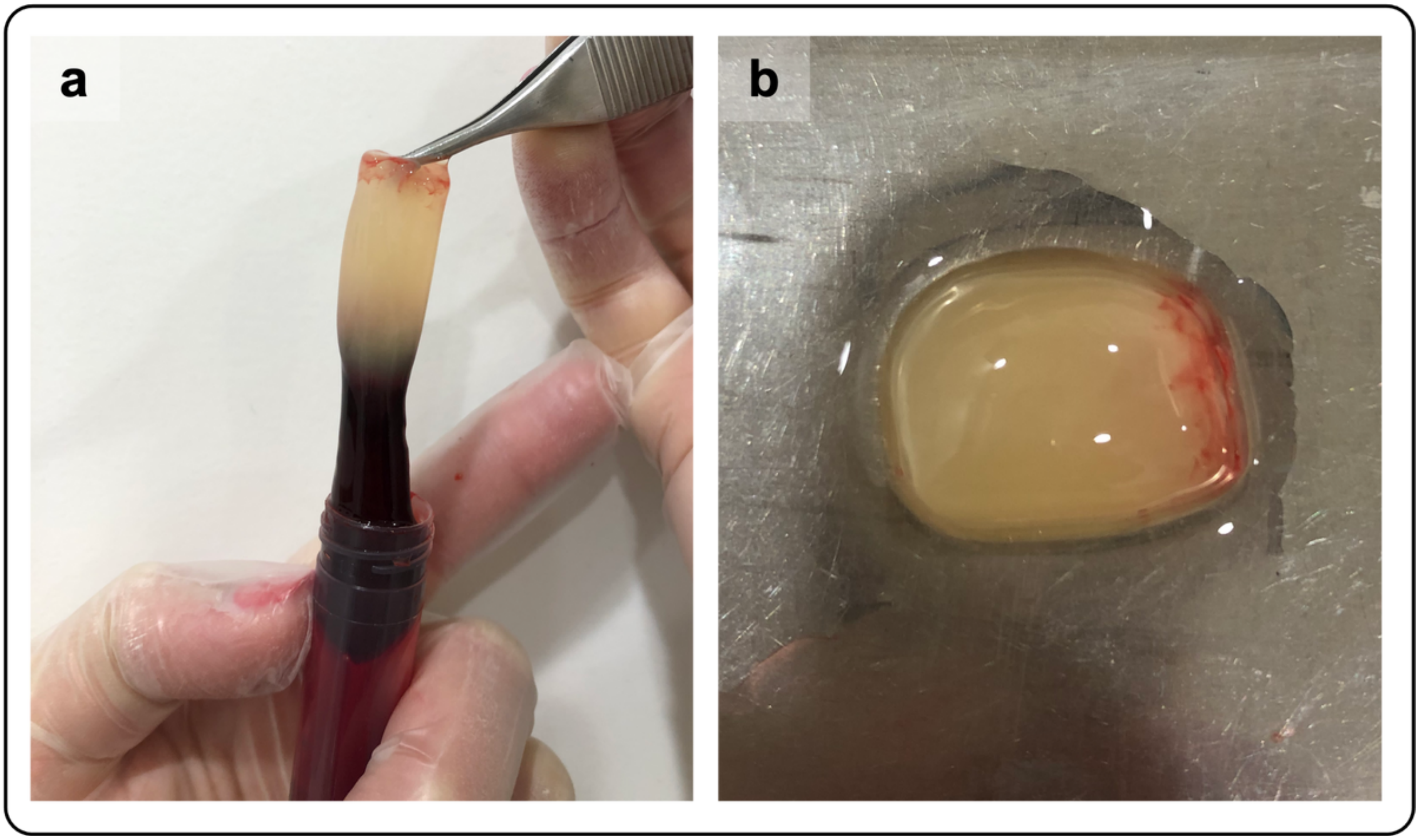

Figure 5

Macroscopic aspect of the PRF clots: a) Clot removal from the tube after its centrifugation and polymerization; b) Clot ready to be grafted, after discarding the red portion.

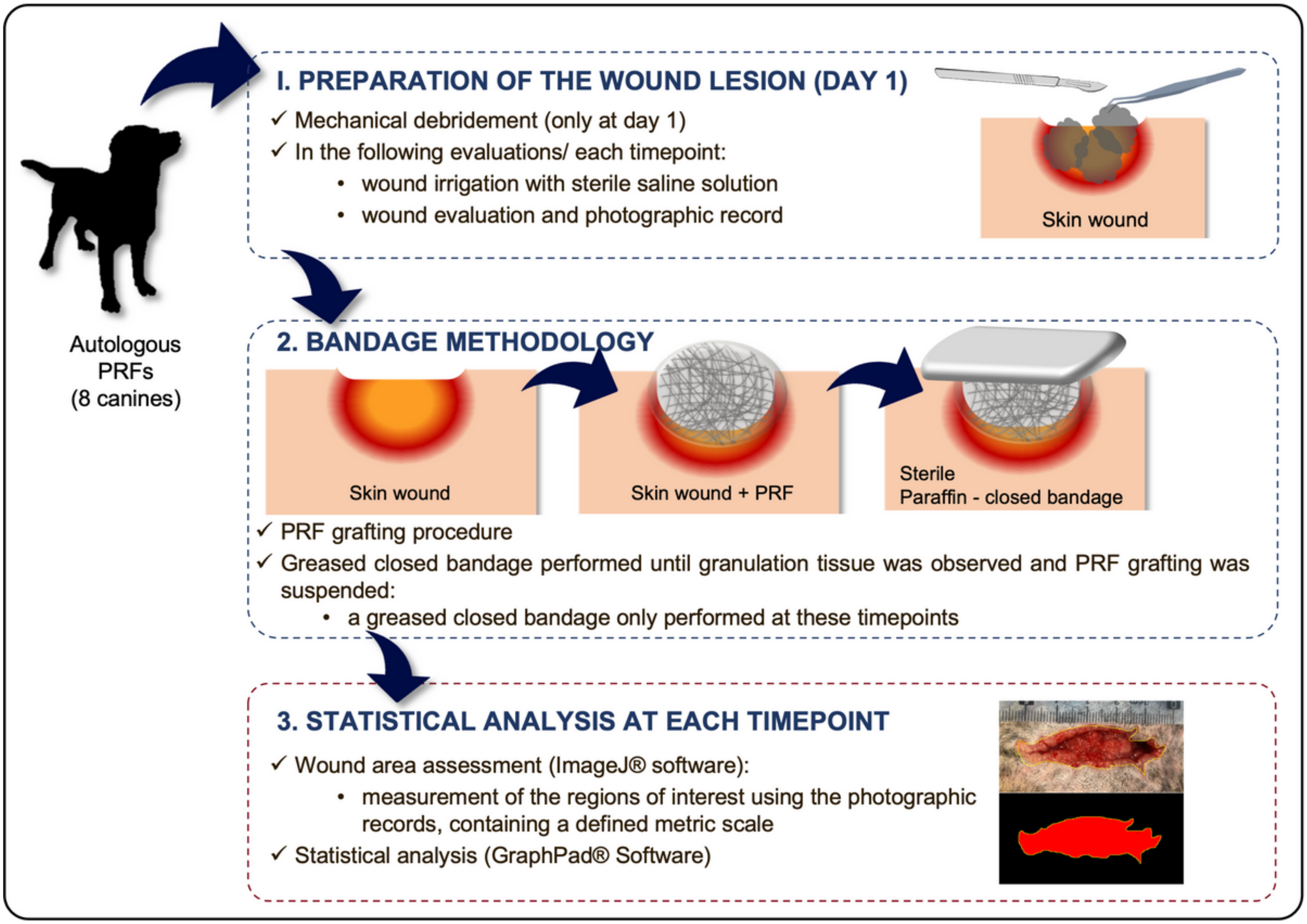

Figure 6

Graphical representation of the PRF application and evaluation.

Supplementary Files

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