

Original research

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PREPARATION AND *IN VITRO* WOUND HEALING EFFECTS OF ANIMAL-BASED PLATELET-RICH PLASMA LYSATE

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Abstract

Wound healing is a vital field of study in both human and veterinary medicine, particularly in the treatment of chronic wounds. Platelet-rich plasma, known for its potential to enhance tissue regeneration due to its high concentration of growth factors, can be further processed into Platelet-rich plasma lysate. As an acellular product, Platelet-rich plasma lysate may exhibit reduced immunogenicity and can be stored frozen for future applications. Using heterologous Platelet-rich plasma lysate derived from larger animal species can be beneficial, particularly for veterinary patients who are unable to provide their own blood due to conditions such as low body weight, coagulopathy, or anemia. This study aims to evaluate and compare

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the *in vitro* wound-healing effects of Platelet-rich plasma lysate derived from equine, porcine, and caprine sources on dermal fibroblasts. Platelet-rich plasma lysate was prepared using a double centrifugation method and freeze-thaw protocol, and its effects were assessed through cell viability and *in vitro* wound healing assays. The cell viability assay revealed a positive dose-dependent effect of Platelet-rich plasma lysate from all tested animal species. Notably, 10% and 20% Platelet-rich plasma lysate from caprine and equine sources, as well as 20% caprine Platelet-rich plasma lysate, showed significantly higher cell viability compared to the negative control ($p < 0.05$). The *in vitro* wound healing assay showed that all Platelet-rich plasma lysate from the selected species had positive cell migration effects on dermal fibroblasts comparable to the positive control (10% Fetal Bovine Serum) at 5% concentration. However, a negative dose-dependent effect followed at higher concentrations. When compared to negative control, only positive control (10% Fetal Bovine Serum), 5% porcine Platelet-rich plasma lysate, as well as 5% and 10% caprine Platelet-rich plasma lysate were found to have significantly higher cell migration effect on dermal fibroblasts at 48-hour post-scratch. These findings highlight the potential of animal-derived Platelet-rich plasma lysate particularly of caprine origin, as an alternative treatment for wound healing, though further *in vivo* studies are needed to confirm its clinical applicability.

Key words: blood cells, fibroblasts, blood plasma, platelets, healing

PRIPREMA I *IN VITRO* EFEKTI LIZATA BOGATOG TROMBOCITIMA POREKLOM OD ŽIVOTINJA NA ZARASTANJE RANA

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Kratak sadržaj

Zarastanje rana predstavlja važno područje istraživanja kako u humanoj, tako u i veterinarskoj medicini, sa posebnim fokusom na lečenje hroničnih rana. Plazma bogata trombocitima, poznata po svom potencijalu da poboljša regeneraciju tkiva zahvaljujući visokoj koncentraciji faktora rasta, može se dalje preraditi u lizate plazme bogate trombocitima. Kao acelularni proizvod, lizat plazme bogate trombocitima može imati smanjenu imunogenost i može se čuvati zamrznut za buduću upotrebu. Korišćenje heterolognog lizata plazme bogate trombocitima dobijene od većih životinjskih vrsta može biti korisno, naročito kod veterinarskih pacijenata koji nisu u mogućnosti da daju sopstvenu krv zbog male telesne mase, koagulopatije ili anemije. Cilj ovog rada je da proceni i uporedi *in vitro* efekte zarastanja rana lizatom plazme bogate trombocitima dobijenog iz konjske,

svinjske i kozje plazme na dermalne fibroblaste. Lizat plazme bogate trombocitima je pripremljen pomoću metode dvostruke centrifuge i protokola zamrzavanja i odmrzavanja, a njegovi efekti su procenjeni putem testova ćelijske održivosti i *in vitro* zarastanja rana. Test ćelijske održivosti je pokazao pozitivan, i od doze zavisian efekat lizata plazme bogate trombocitima kod svih ispitivanih životinjskih vrsta. Značajno je da su 10% i 20% lizata plazme bogate trombocitima poreklom od koza i konja, kao i 20% kozji lizat plazme bogate trombocitima, pokazali značajno višu ćelijsku održivost u poređenju sa negativnom kontrolnom grupom ($p < 0,05$). *In vitro* test zarastanja rana pokazao je da su svi lizati plazme bogate trombocitima iz odabranih životinjskih vrsta imali pozitivan efekat na migraciju dermalnih fibroblasta, uporediv sa pozitivnom kontrolom (10% fetalni goveđi serum) pri koncentraciji od 5%. Međutim, pri višim koncentracijama zabeležen je negativan, od doze zavistan, efekat. U poređenju sa negativnom kontrolom, samo pozitivna kontrola (10% fetalni goveđi serum), 5% svinjski lizat plazme bogate trombocitima, kao i 5% i 10% kozji lizat plazme bogate trombocitima imali su značajno veći efekat na migraciju dermalnih fibroblasta 48 sati nakon nastanka ogrebotine. Ovi rezultati ističu potencijal lizata plazme bogate trombocitima životinjskog porekla, naročito onog kozjeg porekla, kao alternativnog tretmana za zarastanje rana, iako su dalja *in vivo* istraživanja neophodna kako bi se potvrdila njegova klinička primenljivost.

Ključne reči: krvne ćelije, fibroblasti, krvna plazma, trombociti, zarastanje

INTRODUCTION

Wound healing is extensively researched in both human and veterinary medicine because of its clinical importance, scientific relevance, and economic impact (Abegão et al., 2015; Farghali et al., 2017), despite the availability of numerous treatment options (Tanimu et al., 2022). In veterinary practice, skin wounds are among the most commonly encountered conditions (Kožár et al., 2018). These wounds can often progress to chronic conditions, caused by various factors like infection, certain medications, metabolic diseases, nutritional deficiencies and radiation therapy (Lux, 2022). Many complications can arise from having chronic wounds as it may not only lead to prolonged hospitalization and high financial burden, but could also lead to infection and worse, limb amputations (Järbrink et al., 2016). In wound healing management, if

regeneration remains incomplete despite all efforts, adjunct therapies such as platelet-rich plasma (PRP) may be considered (Lux, 2022; Harries et al., 2016).

PRP is a plasma fraction enriched with platelets, obtained by separating plasma from whole blood and concentrating the platelets through single or double centrifugation (Chicharro-Alcántara et al., 2018). Research has shown that PRP's potential in tissue regeneration stems from its high platelet concentration, which acts as a reservoir for essential growth factors (Abegão et al., 2015). Clinically, PRP has been used to promote healing in soft tissues, orthopedics, dentistry, ophthalmology, neurology, and chronic skin ulcers (Chicharro-Alcántara et al., 2018; Notodihardjo et al., 2019). PRP can be further activated to release growth factors by adding substances such as thrombin, calcium salts, or collagen, transforming it into platelet gel (PG). Alternatively, it can undergo freeze-thaw cycles or sonication to become platelet lysate (PL) or platelet-rich plasma lysate (PRPL) (Bonferoni et al., 2019). Depending on its source, PRP can be autologous (derived from the same recipient), allogeneic (from a donor of the same species), or heterologous (from a donor of a different species) (Chicharro-Alcántara et al., 2018). As an acellular product, PRPL offers several advantages, including reduced immunogenicity, extended shelf life, and the ability to be stored frozen until use (Gilbertie et al., 2018).

Although limited data suggest that heterologous PRP is safe and does not cause adverse reactions, the use of heterologous platelet derivatives remains insufficiently explored. Among these data were the use of heterologous canine PRP in contaminated cutaneous wound in a feline case, and *in vivo* study on rabbit wound showing no adverse reactions while promoting tissue healing (Abegão et al., 2015; Gemignani et al., 2017). Heterologous PRPL can be beneficial in cases where patients are unable to donate their own blood, particularly those with small body size or conditions such as anemia or hypovolemic shock. Large animals like ruminants, porcine, or equine, could be selected as potential sources of heterologous PRPL as they have a higher total blood volume, and could be used as an option to treat smaller species of animal like cats and dogs in veterinary field, particularly in cases of delayed or chronic wounds.

This study aims to investigate and compare the *in vitro* wound healing effects using PRPL from equine, porcine, and caprine sources in order to provide an alternative for tissue regeneration. This research will focus on the characterization and effects of the animal-based PRPL dermal fibroblasts as one of the cells involved in skin wound healing. The detailed abbreviations and definitions used in the paper are listed in Table 1.

Table 1. List of abbreviations and definitions used in the paper

Abbreviation	Definition	Abbreviation	Definition
ACD-A	Acid citrate dextrose-A	NHDF	Normal human dermal fibroblasts
CBC	Complete blood count	NC	Negative control
CPPPL	Caprine platelet-rich plasma lysate	PC	Positive control
CPRPL	Caprine platelet-rich plasma lysate	PG	Platelet gel
DMEM/F12	Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12	PL	Platelet lysate
DMSO	Dimethyl sulfoxide	PPP	Platelet-poor plasma
EPPPL	Equine platelet-rich plasma lysate	PPPL	Platelet-poor plasma lysate
EPRPL	Equine platelet-rich plasma lysate	PPPPL	Porcine platelet-rich plasma lysate
FBS	Fetal bovine serum	PPRPL	Porcine platelet-rich plasma lysate
IL-1 β	Interleukin-1 β	PRP	Platelet-rich plasma
PBS	Phosphate buffered saline	PRPL	Platelet-rich plasma lysate
LP-PPP	Leukocyte-poor platelet-rich plasma	RBC	Red blood cells
LR-PRP	Leukocyte-rich platelet-rich plasma	TNF- α	Tumor necrosis factor- α (TNF- α),
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide	WBC	White blood cells

MATERIAL AND METHODS

Blood collection and preparation of PRPL

PRPL was prepared using whole blood collected from three animal species: equine, porcine, and caprine. Blood samples were purchased from the Large Animal and Exotic Unit, University Veterinary Hospital, University Putra Malaysia from a 23-year-old castrated male horse, a 5-month-old intact female pig, and three 1- to 2-year-old intact female goats. Approximately 100 mL of whole blood, either single-source or pooled, was collected from each species. Ethical approval was not required, as the blood was collected during routine veterinary procedures by licensed personnel, following institutional animal welfare guidelines and ensuring that no unethical practices were involved.

The preparation of PRP followed a double-centrifugation protocol adapted and modified from Gilbertie et al. (2018). Blood samples were collected in tubes containing acid citrate dextrose-A (ACD-A) anticoagulant at a 1:9 ratio (anticoagulant: blood). A small aliquot of the anticoagulated whole blood was submitted for a complete blood count (CBC) analysis.

In order to separate plasma from red blood cells, the anticoagulated whole blood was transferred into 15 mL conical tubes and centrifuged (Eppendorf® Centrifuge 5702R, USA) under species-specific conditions: $300 \times g$ at 25°C for 15 minutes for equine and porcine samples, and $1000 \times g$ at 25°C for 30 minutes for caprine samples. Caprine samples required higher centrifugation speed and duration during the first spin because inadequate separation between plasma and red blood cells was observed. The plasma layer was carefully collected and transferred into 50 mL conical tubes, followed by a second centrifugation at $1500 \times g$ at 25°C for 15 minutes to concentrate the platelets. The resulting supernatant, designated as platelet-poor plasma (PPP), was transferred into a new conical tube, leaving approximately 10–15% of the original plasma volume to resuspend the platelet pellet, yielding the initial PRP.

A small aliquot of PRP and PPP were submitted for a haemogram to determine the initial platelet concentration. The PRP was then diluted with PPP accordingly to achieve a standardized platelet concentration of approximately $1 \times 10^6/\mu\text{L}$.

To produce PRPL, the PRP from all species were aliquoted into 1.5 mL centrifuge tubes and subjected to three consecutive freeze-thaw cycles. The samples were frozen at -80°C for 24 hours, thawed in a 37°C water bath for 5–10 minutes, and subsequently refrozen. After the third thaw, the samples were centrifuged (Eppendorf® Centrifuge 5417R, USA) at $20,000 \times g$ for 20 minutes at 4°C . The supernatant was collected and filtered through a $0.22 \mu\text{m}$

sterile syringe filters with polyethersulfone (PES) filter membranes (Microlab Scientific) to eliminate cellular debris and potential microbial contamination. Platelet-poor plasma lysate (PPPL) was also produced by the freeze-thawing PPP in the same manner as PRPL. PRPL and PPPL from the selected animal species were then aliquoted into new 1.5 - 2.0 mL microcentrifuge tubes (Figure 1) and stored at -80°C until further use. The overall procedure is illustrated in Figure 2.

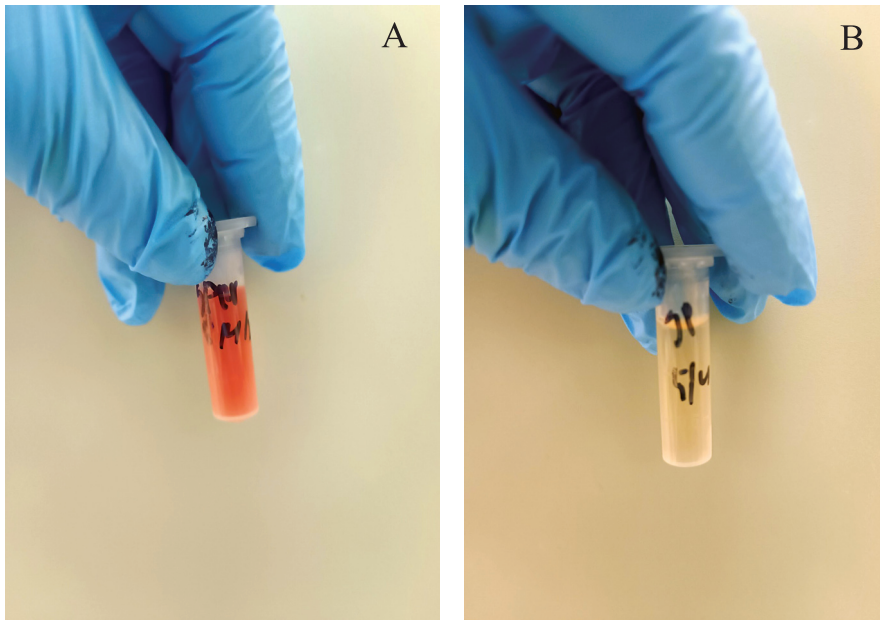


Figure 1. Images showing PRPL (A) and PPPL(B)

Haematological analysis

Haematological analysis was performed on whole blood, PRP, and PPP from the equine, porcine and caprine sources in order to compare red blood cell (RBC), white blood cell (WBC), and platelet concentrations prior to the freeze-thawing and filtration processes used to produce PRPL and PPPL.

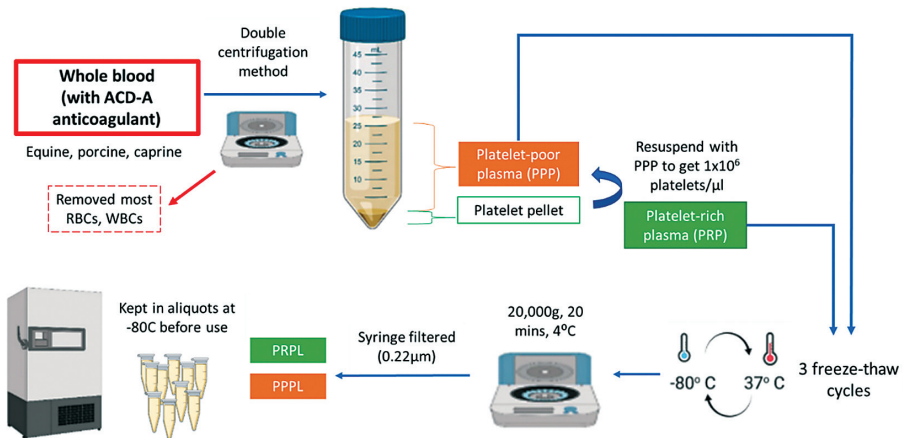


Figure 2. Schematic diagram showing the preparation of PRPL and PPPL.

Cell culture

Normal human dermal fibroblast (NHDF) cells were sourced from the Tissue Engineering Centre at Malaysia Medical Centre, Kebangsaan University, to serve as a representative cell type involved in skin wound healing for this study. The cells were cultured in flasks using Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12, 1:1, 1×; Thermo Fisher Scientific, USA), supplemented with 10% fetal bovine serum (FBS; Thermo Fisher Scientific, USA) and 1% Antibiotic-Antimycotic solution (100×; Thermo Fisher Scientific, USA). The cells were expanded until reaching confluence prior to use in in vitro experiments, with only passages 4 to 6 included throughout the study.

Cell viability assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, a colorimetric technique, was conducted to evaluate the effects of PRPL and PPPL derived from equine, porcine, and caprine sources on the metabolic activity of dermal fibroblasts, as an indicator of cell viability, proliferation, and cytotoxicity (Figure 3). Viable active cells can reduce MTT reagent to formazan. Fibroblasts were seeded at a density of 10,000 cells per well in complete media (DMEM/F12 supplemented with 10% FBS) in 96-well plates and incubated overnight at 37 °C. The following day, cells were treated with media containing 5%, 10%, and 20% PRPL or PPPL from each species. To prevent gel formation, heparinized saline was added to the treatment media at a final concentration of 2 IU/mL, as described by Naskou et al. (2018). Cells cultured

in 10% FBS-supplemented media served as positive controls (PC), while those in serum-free media were used as negative controls (NC). After 24 hours of incubation at 37°C, the treatment media were removed, and 100 µL of serum-free DMEM/F12 along with 10 µL of MTT reagent (5 mg/mL in PBS; Thermo Fisher Scientific, USA) were added to each well. The cells were incubated for an additional 4 hours at 37 °C. Following incubation, the MTT-containing media were carefully aspirated, and 100 µL of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. The plates were placed on an orbital shaker set at 100 rpm for 20 minutes at room temperature. Absorbance was measured at 570 nm using a spectrophotometer, and cell viability was calculated as a percentage relative to the NC using the following equation:

$$\text{Cell viability \%} = \frac{\text{X}}{\text{NC}} \times 100\%$$

Since the denominator in the formula is the mean absorbance of the NC, the calculated cell viability percentage for the NC will be 100%. Any value above or below 100% indicates higher or lower cell viability, respectively, compared to the NC.

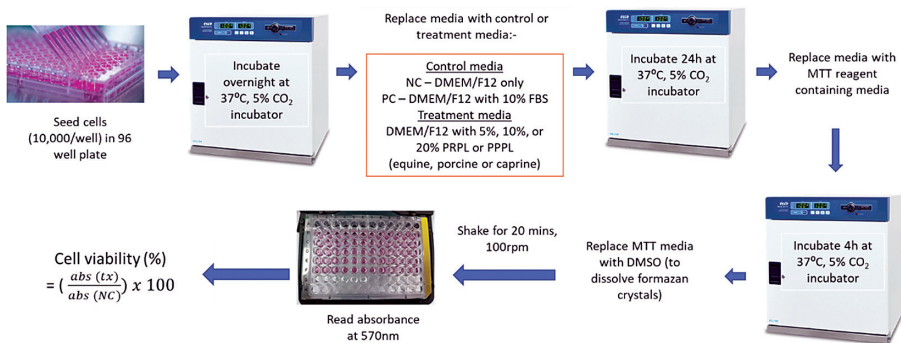


Figure 3. Schematic diagram showing the method of cell viability assay

In vitro wound healing assay

The scratch wound assay was conducted to assess the effects of PRPL and PPPL from different sources on dermal fibroblast migration (Figure 4). Dermal fibroblasts (5×10^4 cells) were seeded into 24-well plates with 500 µL of complete media (DMEM/F12 supplemented with 10% FBS) and cultured until confluence. Once confluent, a linear scratch was created at the centre of each well using an SPL Scar™ Scratcher. The old media were removed, and cells were

washed with phosphate-buffered saline (PBS) to eliminate debris from damaged or dead cells.

Following the wash, 500 µL of treatment media containing 5%, 10%, or 20% PRPL/PPPL were added to the respective wells, alongside control media (10% FBS as a positive control and serum-free media as a negative control). To prevent gel formation, heparinized saline was added to the treatment media at a final concentration of 2 IU/mL, as described by Naskou et al. (2018). The plates were incubated at 37 °C for 72 hours.

Images of the scratch area were captured at 0, 24, 48, and 72 hours post-scratch using a digital camera are attached to an inverted microscope. The scratch areas were measured at each time point using ImageJ software, and the percentage of wound closure was calculated using the following formula:

$$\text{Scratch closure \%} = \frac{\text{Scratch area at } T_0 - \text{Scratch area at } T_x}{\text{Scratch area at } T_0} \times 100\%$$

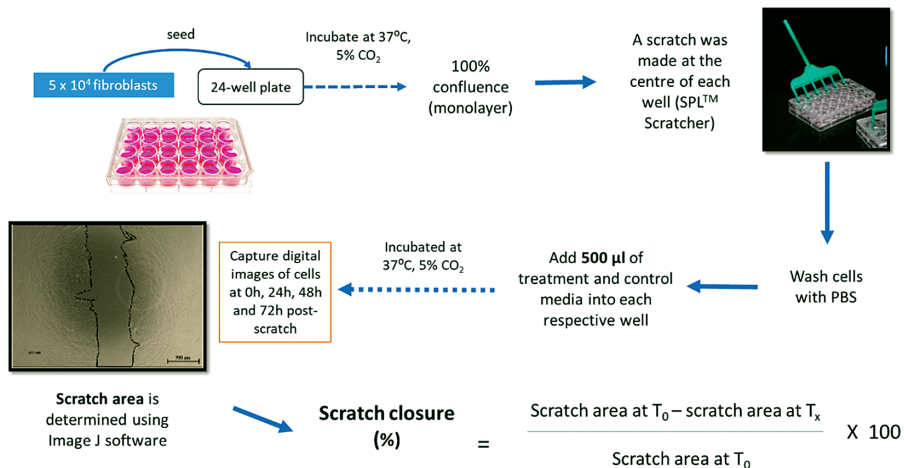


Figure 4. Schematic diagram showing the method of in vitro wound healing assay.

Statistical analysis

All experiments were performed in at least three replicates. Data analysis was conducted using GraphPad Prism (version 9.5.1, USA), employing the Kruskal-Wallis test followed by Dunn’s multiple comparison analysis for the MTT assay and two-way ANOVA with Tukey’s multiple comparison analysis for the scratch wound assay. A *p*-value of less than 0.05 was considered statistically significant.

RESULTS

The haematological results are presented in Table 2. The platelet concentration in the resultant PRP from each animal species was successfully maintained at approximately $1 \times 10^6/\mu\text{l}$, while the RBC and WBC concentrations were lower than those in whole blood. In contrast, PPP from each animal species contained lower concentrations of RBC, WBC, and platelets compared to their respective whole blood (WB) and PRP.

Table 2. Haematological profile of whole blood (WB), platelet-poor plasma (PPP) and platelet-rich plasma (PRP) from equine, porcine, and caprine sources.

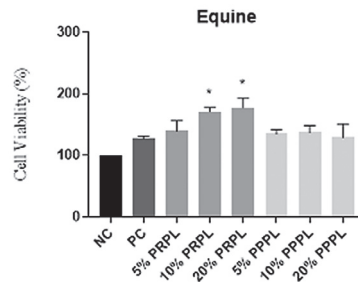
Animals	Samples	RBC ($\times 10^6/\mu\text{l}$)	Reference values ($\times 10^6/\mu\text{L}$)*	WBC ($\times 10^3/\mu\text{l}$)	Reference values ($\times 10^3/\mu\text{L}$)*	Platelet ($\times 10^3/\mu\text{L}$)	Reference values ($\times 10^3/\mu\text{L}$)*
Equine	WB	5.87	6.00–10.40	8.20	5.60–12.10	109.00	117.00– 256.00
	PPP	0.00	-	0.00	-	12.00	-
	PRP	0.01	-	1.50	-	1039.00	-
Porcine	WB	5.61	5.00–8.00	13.90	11.00– 22.00	430.00	200.00– 500.00
	PPP	0.00	-	0.00	-	26.00	-
	PRP	0.02	-	0.20	-	1111.00	-
Caprine	WB	$19.00 \pm$ 1.86	8.00–18.00	11.52 ± 3.92	4.00–13.00	$423.40 \pm$ 223.84	300.00– 600.00
	PPP	$0.01 \pm$ 0.01	-	$0.01 \pm$ 0.01	-	$88.67 \pm$ 21.03	-
	PRP	$0.80 \pm$ 0.43	-	$4.26 \pm$ 1.46	-	1015.33 ± 80.75	-

*Note: The reference ranges in this table are adapted from "Hematology (Complete Blood Count) Reference Ranges," by MSD Veterinary Manual, n.d., <https://www.msddvetmanual.com/multimedia/table/hematology-complete-blood-count-reference-ranges>.

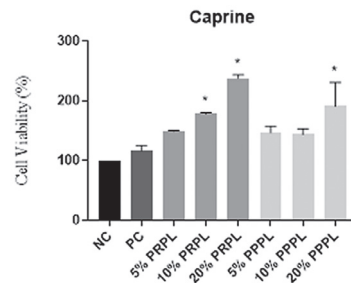
Figure 5. illustrates the cell viability percentages of dermal fibroblasts, as determined by the MTT assay, following treatment with PRPL and PPPL derived from equine, porcine, and caprine sources. A dose-dependent effect was observed, with increasing PRPL concentrations leading to higher cell viability percentages across all selected species. However, statistical analysis revealed that only 10% and 20% PRPL from caprine and equine sources, along with 20% caprine PPPL, demonstrated significantly higher cell viability ($p < 0.05$)

compared to the negative control. Among these, 20% caprine PRPL exhibited the highest cell viability, compared to PRPL from equine and porcine sources. Interestingly, caprine PPPL at 20% concentration demonstrated higher cell viability than equine and porcine PPPL at similar concentration.

A



B



C

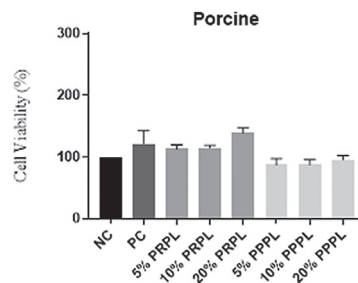
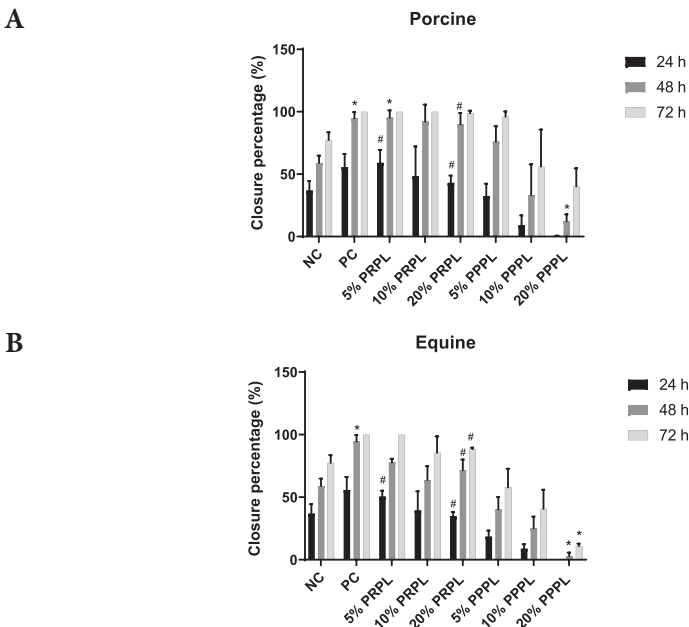


Figure 5. Dermal fibroblasts cell viability after 24 h incubation with control media (negative control (NC), DMEM/F12 only; positive control (PC), DMEM/F12 with 10% FBS) and treatment media (DMEM/F12 with 5%, 10% or 20% PRPL or PPPL from equine (A), porcine (B), or caprine (C) sources). Experiments were completed in at least triplicates, and the values were expressed as mean percentage cell viability \pm SD. * $p < 0.05$ when compared with NC. DMEM/F12, Dulbecco's Modified Eagle Medium; Nutrient Mixture F-12 medium; FBS, fetal bovine serum; NC, negative control; PC, positive control; PRPL, platelet-rich plasma lysate; PPPL, platelet-poor plasma lysate.

Figure 6. illustrates the quantitative results of the *in vitro* wound healing assay, presenting the closure percentages, while Figure 7 provides representative micrographs of cell migration within the assay. Compared to the NC, only PC, 5% porcine PRPL, and both 5% and 10% caprine PRPL demonstrated a significantly enhanced cell migration effect on dermal fibroblasts at 48 hours post-scratch. A general negative dose-dependent effect on cell migration was observed as the concentration of PRPL from equine, porcine, and caprine sources increased from 5% to 20%. This trend was particularly evident at 24-hours post-scratch, with the optimal migratory effect consistently observed at the 5% PRPL concentration. A similar dose-dependent pattern was also noted for the corresponding PPPL groups. The highest concentration (20%) of equine PPPL resulted in significantly lower closure percentages than NC at both 24- and 48-hours ($p < 0.05$). A similar significant reduction ($p < 0.05$) was also found in the 20% porcine PPPL group when compared to NC at 48-hours post-scratch. When comparing PRPL to PPPL within the same species and at identical concentrations, the PRPL groups generally exhibited a higher wound closure percentage, especially at 24- and 48- hours post-scratch.



C

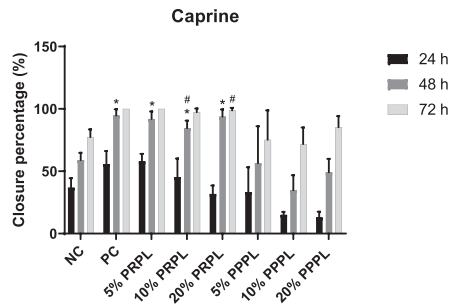
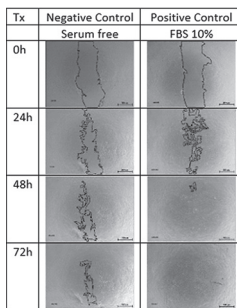
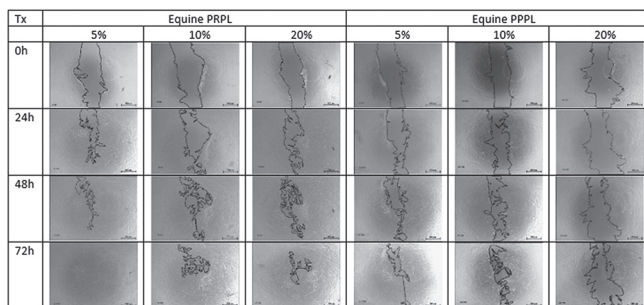


Figure 6. Closure percentage result of the *in vitro* wound healing assay. The graphs show the effect of control media (negative control (NC), DMEM/F12 only; positive control (PC), DMEM/F12 with 10% FBS) and treatment media (DMEM/F12 with 5%, 10% or 20% PRPL or PPPL from equine (A), porcine (B), or caprine (C)) at 24-, 48- and 72-hours post-scratch on the dermal fibroblasts' migration to close the wound area. Experiments were completed in at least triplicates, and the values were expressed as mean percentage cell viability \pm SD. * $p < 0.05$ when compared with NC. # $p < 0.05$ when the same concentration PRPL is compared with PPPL at the specific time-point. DMEM/F12, Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 medium; FBS, fetal bovine serum; h, hour; NC, negative control; PC, positive control; PRPL, platelet-rich plasma lysate; PPPL, platelet-poor plasma lysate.

A



B



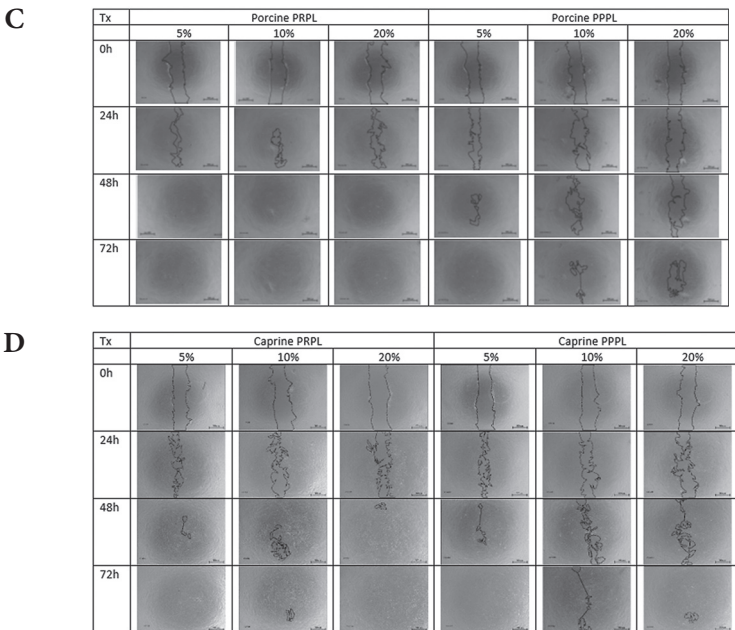


Figure 7: Micrographs of migrating cells in scratch wound assay. The micrographs show the effect of controls (A) (negative control (NC) and positive control (PC)) and different concentrations (5%, 10%, and 20%) of PRPL and PPPL from equine (B), porcine (C), and caprine (D) sources at 0-, 24-, 48- and 72-hours post-scratch on scratch closure of dermal fibroblasts. CPPPL, caprine platelet-poor plasma lysate; CPRPL, caprine platelet-rich plasma lysate; EPPPL, equine platelet poor plasma lysate; EPRPL, equine platelet-rich plasma lysate; h, hour; NC, negative control; PC, positive control; PPPL, platelet-poor plasma lysate; PRPL, platelet-rich plasma lysate; porcine platelet-poor plasma lysate; PPRPL, porcine platelet-rich plasma lysate.

DISCUSSION

Equine, porcine, and caprine sources were selected for this study because they are domestic animals with larger body sizes compared to smaller animals like cats and dogs. Their larger blood volume makes them more suitable for blood collection for the production of heterologous PRPL. Acid citrate dextrose-A (ACD-A) was used as the anticoagulant, as it is commonly employed in the PRP processing and is known to effectively preserve platelet integrity while preventing spontaneous activation, outperforming anticoagulants like heparin (Lei et al., 2009). To produce PRPL and PPPL lysates, three freeze-thaw cycles were used, based on findings by Strandberg et al. (2017), which suggest that three to five cycles are sufficient to release an optimal level of growth factors,

with additional cycles offering minimal clinical benefit. For sterilization, 0.22 µm sterile syringe filters with polyethersulfone (PES) filter membranes (Mircrolab Scientific) were used to remove cellular debris (Gilbertie et al., 2018) and eliminate potential microbial contaminants from the lysates.

PRP is generally characterized by a platelet concentration approximately 3 to 4 times higher than the baseline (Kaneps, 2023). Although the minimum acceptable platelet concentration in PRP is said to be 200,000 platelets/µl (Kaneps, 2023), achieving a platelet concentration of $1 \times 10^6/\mu\text{l}$ may be necessary to elicit the desired regenerative effect (Conde-Montero et al., 2017; Tambella et al. 2018). In the study, PRP prepared from equine, porcine and caprine sources following the established protocol, consistently yielded a platelet count of approximately $1 \times 10^6/\mu\text{L}$ (Table 2). Furthermore, the concentrations of RBCs and WBCs in all PRP samples were found to be at lower than their respective concentrations in whole blood. Although caprine PRP in this study exhibited relatively higher WBC and RBC counts compared to equine and porcine PRP, these counts remained below the baseline whole blood levels. The elevated WBC and RBC content observed in caprine PRP is likely attributable to challenges in achieving distinct separation between plasma and the red blood cell layer, even when employing higher centrifugation speed and duration (1000 g, 30 mins) during the first centrifugation of the whole blood. This is contrast to equine and porcine whole blood that had easier separation of plasma and RBCs at a lower centrifugation speed and duration (300 g, 15 mins). This suggests that during the initial transfer of caprine plasma into a new conical tube after the first centrifugation step, a greater proportion of WBCs and RBCs may have been inadvertently included before the subsequent spin designed to further concentrate the cellular components for PRP production.

Regarding the leukocyte content, PRP can be broadly classified as either leukocyte- rich PRP (LR-PRP) or leukocyte-poor PRP (LP-PRP). This classification is based on whether the leukocyte count in the PRP is higher or lower than the baseline level found in whole blood (Chun et al. 2020). Since all PRP samples in this study, including the caprine PRP, exhibited WBC and RBC counts below baseline levels, they are classified as LP-PRP. This classification applies before any freeze-thaw cycles, which would subsequently convert them into PRPL.

The impact of leukocytes within PRP on tissue healing is a subject of an ongoing debate among researchers. Some authors advocate for including leukocytes in PRP, citing their potential immunological and antibacterial benefits that may enhance healing (Tambella et al., 2018). Conversely, others avoid leukocyte-rich PRP (L-PRP) due to its content of pro-inflammatory and catabolic

factors, such as interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α), which may provoke unwanted inflammation and impair tissue regeneration (Chun et al. 2020). Similarly, RBC levels were kept low, as RBCs contain molecules such as plasma-free haemoglobin, iron, and hemin, which have been associated with cytotoxicity, oxidative stress, and inflammatory responses (Gupta et al., 2023).

The increase in cell viability observed with increasing PRPL concentrations in this study aligns with findings by Russell and Koch (2016), which demonstrated a strong cell viability effect of equine PRPL on equine cord blood mesenchymal stromal cells. However, cell viability declined at concentrations exceeding 30% equine PRPL, whereas the response to FBS continued to increase (Russell and Koch, 2016). Interestingly, caprine PRPL demonstrated superior cell viability effect compared to both equine and porcine PRPL. Similarly, caprine PPPL also outperformed its equine and porcine counterparts. This may suggest that caprine PRPL and PPPL may contain a better composition of bioactive components that contribute to the enhanced healing effects on cells. However, this finding requires further validation, as individual variability in PRPL and PPPL batches may also contribute to the observed differences.

The *in vitro* wound healing assay showed that lower concentration of PRPL (5%) increased cellular migration of dermal fibroblasts than higher concentrations (20%). The adverse effect of higher PRPL concentrations on the cell migration is consistent with the findings by Xian et al. (2015), which reported a significant reduction in fibroblast migration rates at 20% PRP compared to 10% PRP at 48- and 72-hours post-scratch. While the exact mechanism remains unclear, it has been suggested that excessive growth factor concentrations in PRP may downregulate cell receptors via a negative feedback loop (Berndt et al., 2019).

To the authors' knowledge, direct comparative studies on PRPL across different animal species are limited, as most existing research focuses on individual species. Equine PRPL have been extensively investigated for their therapeutic applications, particularly in the context of osteoarthritis in horses (Gilbertie et al., 2018; Perrone et al., 2020), and for the expansion of equine mesenchymal stem cell (Russell and Koch, 2016). Porcine PRPL has shown promise in studies related to wound regeneration (Low et al., 2025) and osteoarthritis (Xiao et al., 2024) in rat models. Furthermore, porcine PRPL has been successfully used as a growth supplement for various animal cell cultures, including Vero (African green monkey kidney epithelial cells), Chinese hamster ovary (CHO) cells, and hybridoma cells, demonstrating efficacy comparable to that of fetal bovine serum (FBS) (Aldén et al., 2007). Information regarding the use of caprine PRPL is relatively scarce, with existing literature primar-

ily focusing on other forms of platelet derivatives. For instance, caprine PRP has been utilized in osteoarthritis studies involving goat models (Wang et al., 2018), while caprine platelet-rich plasma gel has been applied in wound healing research (Khalaf and Salih, 2018) and bone healing in goats (Ferdousy et al., 2014). Given that PRPL offers distinct advantages over other platelet derivatives - such as its acellular nature and suitability for prolonged frozen storage (Gilbertie et al., 2018) - there is considerable potential for further investigation into the use of caprine PRPL in wound regeneration.

CONCLUSION

Based on the study results, PRPL derived from equine, porcine, and caprine sources demonstrated wound-healing potential *in vitro* on dermal fibroblasts. Among them, caprine PRPL appeared to have a superior healing effect compared to equine and porcine PRPL. However, since *in vitro* studies do not fully replicate the complex conditions of the skin, further *in vivo* research is required in order to evaluate the efficacy of animal-derived PRPL in wound healing.

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Author's Contribution:

ARR and SNB contributed to the conception and design of the study, data acquisition, and manuscript revision. CHC and RR were involved in the conception and design. MFWC contributed to data acquisition. AMJ, PJT, and MA participated in the study conception and design and provided final approval of the manuscript.

Competing interest

The author(s) declare that they have no competing interests regarding the publication of this paper.

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