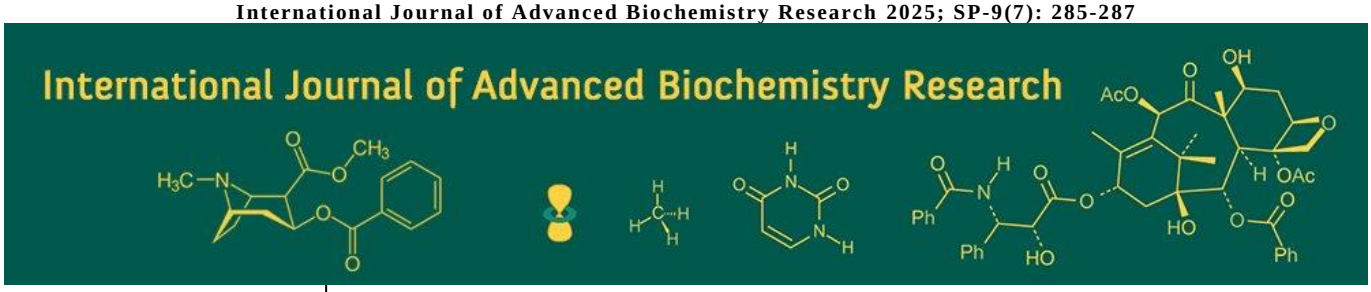

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Preparation and lyophilization of caprine platelet rich fibrin

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Abstract
Platelet Rich Fibrin is evolving as a vital biomaterial for wound management for its cocktail of growth factors. This study was taken to prepare Platelet Rich Fibrin (PRF) from caprine blood, lyophilize it and to study its macroscopic appearance and yield. Blood samples were collected from 20 goats which were centrifuged immediately to prepare the PRF clot. The PRF clot was then lyophilized to obtain lyophilized PRF (LyPRF). The fresh and lyophilized yield of PRF was standardized. The average yield of fresh PRF and LyPRF were 2522.23±169.58 and 184.40±15.19 /10ml of blood respectively. There was more than 90% reduction in the weight of PRF clot during lyophilization. Thus, this study validates a protocol for preparation, lyophilization and standardization of yield from caprine blood that could be used as a heterologous platelet product in regenerative medicine.

Keywords: PRF, caprine, lyophilization

Introduction
Platelet concentrates are widely used to promote wound healing in human and veterinary practice. (Soares *et al.*, 2021, Soares *et al.*, 2024) [2, 3, 6]. Platelets participate in the different stages of wound healing such as angiogenesis, inflammation and tissue remodelling. Platelet possess supraphysiological doses of more than 1500 bioactive factors which plays vital roles in tissue regeneration (Pavlovic *et al.*, 2021) [7]. Several studies have demonstrated the ability of growth factors found in platelets to promote wound healing (Yaprak *et al.*, 2018) [8]. The initial platelet concentrate used was the fibrin glue which was prepared from donor plasma and polymerized to form a surgical additive upon addition of calcium or thrombin. However, its use was limited owing to the risk of disease transmission and high cost of production (Prakash *et al.*, 2011) [9]. In 1998, Platelet Rich Plasma (PRP), a first generation platelet product was introduced. It is obtained by double centrifugation of citrated blood. The PRP formed is then activated with either bovine thrombin or calcium which induce degranulation of platelets and release of growth factors. However, the major drawback of PRP therapy was the burst release of growth factors in the first 10 minutes following administration. Majority of the growth factors (95%) are released during the first hour of administration whereas a sustained release of growth factors is more favourable for wound healing. Moreover, the anticoagulant added to the blood was found to interfere with coagulation and delay wound healing (Shariati *et al.*, 2020) [10]. Platelet Rich Fibrin (PRF), a second generation platelet product was first introduced by Choukraun and his team in France (Dohan *et al.*, 2006) [11]. PRF is prepared by a single step centrifugation of blood soon after collection. The fibrinogen in blood polymerises to fibrin during centrifugation and traps platelets and leukocytes within its mesh. The release of growth factors unlike PRP is not spontaneous, but progressive over time (Pavlovic *et al.*, 2021) [7]. Soares *et al.*, 2021 [2, 3] confirmed sustained release of growth factors including PDGF-BB, TGF-β1 and VEGF-A from canine and feline PRF upto 10 days. However, blood collection is challenging in patients with concomitant haematological and metabolic disorders, excessive blood loss and in animals with reduced blood volume such as cats and miniature dogs. In such cases, xenologous PRF can be considered a significant alternative (Soares *et al.*, 2024) [6]. The effect of xenologous PRF such as dog PRF in cats, buffalo PRF in dogs and cow PRF in rats have already been studied and were reported to positively affect wound healing.

In the present study goat PRF was evaluated considering its easy availability. The other major challenge of PRF therapy is that they are intended for same day application which limits its clinical utility. Lyophilization of the PRF clot by sublimation and subsequent removal of water vapour can drastically increase the shelf life of PRF clot. In the present study lyophilized PRF clot is prepared using caprine blood. The morphological characteristics and yield of fresh and lyophilized PRF were studied.

Materials and Methods

Sample collection: 20 non-descriptive goats were taken for the study. The site of blood collection was aseptically prepared by rubbing with 70% isopropyl alcohol. 10 ml of blood was collected from jugular vein of goats using a 18-gauge needle in a 15 ml sterile polypropylene tube without anticoagulant.

PRF preparation: The steps involved in the preparation of LyPRF are given in Fig.1. PRF was prepared according to the protocol of Soares *et al.*, 2021 [2, 3] with slight modification. The blood samples were centrifuged immediately after collection in a benchtop centrifuge (Remi R-4C Centrifuge Machine) at 2500 rpm for 15 mins at room temperature. After centrifugation the blood separated into 3 layers namely the bottom layer of packed RBCs, middle layer of fibrin clot and an acellular plasma layer at top. The middle fibrin clot was carefully removed with sterile forceps and scissors and stored in a sterile sample container at-80 °C.

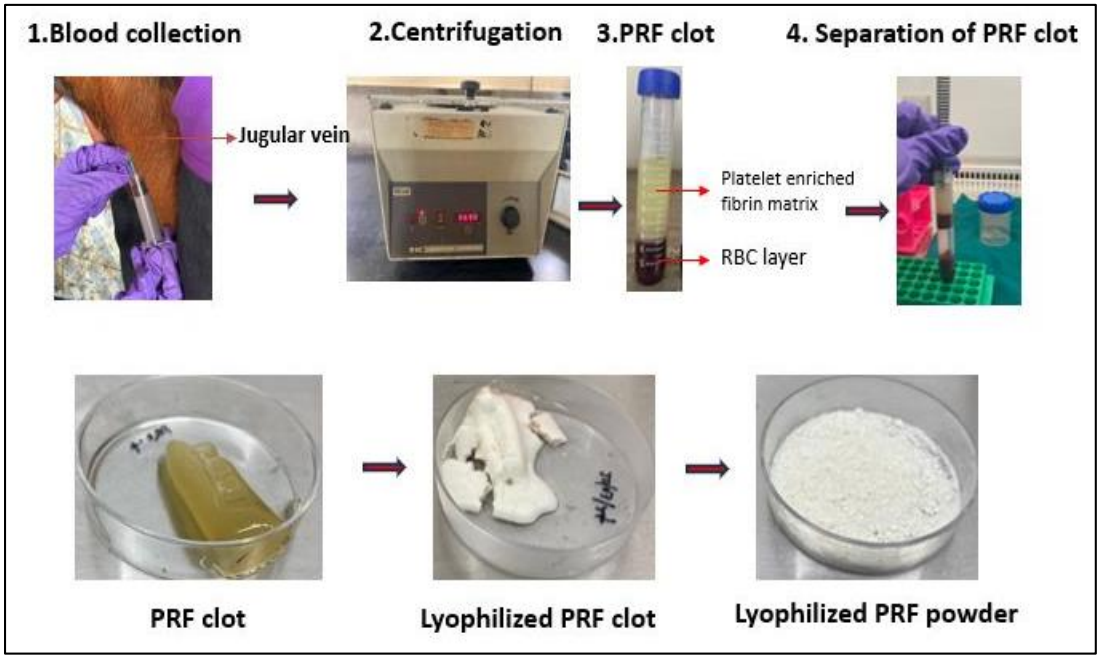

Fig 1: Preparation of Ly-PRF

Lyophilization of PRF clot: Lyophilization of the PRF clot was done by a modified protocol of Ngah *et al.*, 2021 [12] in human PRF. The fresh PRF clot was frozen at-80 °C overnight prior to lyophilization. The PRF clot along with the exudate was then lyophilized at-51 °C for 18 hrs in 25 L Genesis SQ EL-85 Lyophilizer, SP Scietific. The LyPRF was stored at-20 °C until use.

PRF yield: The average yield of Fresh and LyPRF from 10 mL of whole blood was calculated using the formula

$$\text{Fresh PRF yield (\%)} = \frac{\text{Mean weight of fresh PRF clot}}{\text{Mean weight of whole blood}} \times 100$$

$$\text{LyPRF yield (\%)} = \frac{\text{Mean weight of lyophilized PRF}}{\text{Mean weight of whole blood}} \times 100$$

The lyophilization yield of the PRF clot was calculated as follows

$$\text{Lyophilization yield (\%)} = \frac{\text{Mean weight of Lyophilized PRF clot}}{\text{Mean weight of fresh PRF clot}} \times 100$$

Statistical Analysis: The data was analysed using appropriate statistical measures and the results are expressed as mean ± standard error.

Results and Discussion

PRF clot analysis: Centrifugation of whole blood at 2500 rpm for 15 mins resulted in separation of blood into 3 layers namely, the bottom RBC layer, middle PRF layer and upper layer of acellular plasma. The caprine PRF clot was white-creamy in colour and had a gel like consistency. The ratio of PRF clot to RBC layer was 1.5:1. After lyophilization, the PRF clot resembled a white porous structure. Banyatworakul *et al.*, 2021 [1] reported a PRF to RBC ratio of 1:1 in Thai and Murrah buffalo blood. Soares *et al.*, 2021 [2, 3] characterized PRF clot obtained from canine and feline origin and reported similar findings in terms of PRF colour and consistency. The short shelf life of fresh PRF clot limits its application under clinical settings. Hence in the present study, lyophilization of the PRF clot was done to increase the storage time and produce an off the shelf product. Macroscopically, the PRF clot resembled a white spongy structure similar to human Ly-PRF described by Ngah *et al.*, 2021 [12].

Average yield: The average weight of fresh and lyophilized PRF are given in table 1. The maximal weight recorded for the fresh PRF clot was 3100 mg and the minimal weight recorded was 1472 mg. For the lyophilized PRF clot the maximal weight was 294 mg and the minimal weight was

150 mg. The average weight of fresh PRF and lyophilized PRF from 10 ml of whole blood was 2522.23±169.58 and 184.40±15.19 respectively. The results were consistent with previously reported findings, indicating that the average weight of lyophilized PRF prepared from 10 mL of human blood was 262 mg and 150 mg, respectively (Ngah *et al.*, 2021; Anthraper *et al.*, 2024) [12, 4]. The Ly-PRF yield and the lyophilization yield of the PRF clot from 10 ml whole blood was 1.69±0.13% and 7.42±0.52% respectively. More than 90% reduction in weight loss was noticed in the Ly-PRF clot when compared to fresh PRF. The results aligned with the findings of Ngah *et al.*, 2021 [12] who reported a weight loss of 70-91% in fresh PRF following lyophilization.

Table 1: Mean weight, yield and moiture of fresh and lyophilized PRF

Parameter	Mean ± SE
PRF wet weight (mg)	2522.23±169.58
LyPRF weight (mg)	184.40±15.19
Fresh PRF yield (%)	23.22±1.45
LyPRF yield (%)	1.69±0.13
Lyophilization yield (%)	7.42±0.52
Moisture loss (%)	92.58±0.52

In conclusion, this study puts forward an effective protocol for the preparation and lyophilization of PRF obtained from caprine blood. Moreover, the study also standardized the average yield of LyPRF from whole blood which is highly essential in formulating treatment plans. Further studies involving the clinical application of the LyPRF clot for wound healing.

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