

Review

# Standardization of Animal Models and Techniques for Platelet-Rich Fibrin Production: A Narrative Review and Guideline

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**Abstract:** Experimental research is critical for advancing medical knowledge and enhancing patient outcomes, including in vitro and in vivo preclinical assessments. Platelet-rich fibrin (PRF) is a blood by-product that has garnered attention in the medical and dental fields due to its potential for tissue regeneration and wound healing. Animal models, such as rabbits and rats, have been used to produce PRF and examine its properties and applications. PRF has demonstrated potential in the dental and medical fields for reducing inflammation, promoting tissue repair, and accelerating wound healing. This narrative review aims to compare existing evidence and provide guidelines for PRF animal research, emphasizing the importance of standardizing animal models, following ethical considerations, and maintaining transparency and accountability. The authors highlight the necessity to use the correct relative centrifugal force (RCF), standardize centrifugal calibration, and report detailed information about blood collection and centrifuge parameters for reproducible results. Standardizing animal models and techniques is crucial for narrowing the gap between laboratory research and clinical applications, ultimately enhancing the translation of findings from bench to bedside.

**Keywords:** platelet-rich fibrin; animal models; experimental research; guideline



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## 1. Introduction

Translational research is a critical process that involves the translation of basic scientific discoveries into practical applications that benefit human health [1]. It consists of bridging the gap between basic research and clinical practice by using experimental studies to develop new techniques and procedures in the medical field. Experimental studies play a crucial role in the development of new medical techniques and procedures, as they provide the necessary evidence to support the effectiveness and safety of these innovations. For example, clinical trials are essential to testing the efficacy of new treatments and drugs before they are approved for use in patients. Advancing medical knowledge and developing new treatments that improve patient outcomes would be impossible without experimental studies, including important results derived from in vitro and in vivo pre-clinical assessments. The presence of experimental research in translational research is a

vital step in ensuring that scientific discoveries are translated into practical applications that improve human health [2,3].

In the past two decades, platelet-rich fibrin (PRF) has become increasingly popular in medicine and dentistry for its potential use in wound healing and tissue regeneration [4–8]. However, PRF's development deviated from the typical research process as it was first used in humans before being studied *in vitro* and with animal models [9]. This unconventional approach was taken to enhance the research and gain a better understanding of the blood by-product. This sequence of application was likely due to the urgent need for effective treatments in human patients, leading to the rapid adoption of PRF in clinical practice. As more knowledge and experience were gained, researchers began to study PRF *in vitro* and in animal models to better understand its mechanisms of action and optimize its use [10,11]. Such studies have yielded valuable insights into the properties and potential applications of PRF, paving the way for further translational research to develop new techniques and procedures [12–15].

In order to improve the techniques and discover new clinical applications, animal models, such as rabbits [16–20] and rats [21–23], have been used to produce PRF and investigate its properties and potential applications. The high concentrations of platelets, growth factors, and other bioactive molecules make PRF useful in promoting tissue regeneration and enhancing bone growth [11]. In the dental field, the blood by-product has been used in various procedures to reduce inflammation, accelerate wound healing, and improve outcomes. In the medical field, PRF has been used to treat chronic wounds, burns, and musculoskeletal injuries, with promising results in enhancing tissue repair and reducing inflammation [24,25].

Fibrin research has shown that fibrin clots in mammals such as mice, rats, and rabbits can exhibit different fiber diameters and clot densities compared to human clots. These differences have been considered when using animal models to study blood clotting and related disorders in humans, as they can inform therapeutic development and enhance our understanding of blood clotting mechanisms in general [26,27].

Compared to platelet-rich plasma (PRP), PRF does not necessitate the addition of anticoagulants or other external factors during its preparation. This simplifies the process and potentially mitigates the risk of adverse effects related to additives [27]. Nevertheless, the anticoagulants in PRP can aid researchers in acquiring the appropriate amount of this blood derivative for use in animal models, giving them more time during the blood draw.

The major concern about PRF's production using animal models is the necessity to have fast blood collection, which is essential in producing high-quality PRF as it minimizes the risk of clotting and ensures that the concentration of platelets and other bioactive molecules remains high. However, in some animal species, such as rabbits and rats, blood coagulates quickly, making it necessary to collect blood rapidly to avoid clotting. In addition, tubes with clot activators, such as silica, can also aid in blood coagulation and make PRF's production difficult. In addition, access to appropriate vessels (veins or arteries) from animals can be challenging, and the amount of blood that can be obtained may be limited. In this context, PRF has been investigated through very diverse animal models with a great heterogeneity of methodologies, with little help available for the choice of the best protocol for the production of this blood byproduct.

The aim of this narrative review is to compare the available evidence and provide a helpful guideline for animal research on PRF. The guideline aims to assist researchers in avoiding unnecessary loss of time and taking the lives of animals during the study process. It is essential to emphasize the importance of animal welfare and ethical considerations in research, and this guideline serves to clarify and guide future studies in this area.

## 2. Definitions and Search Process

Experimental studies aim to investigate the properties and potential applications of PRF. For this narrative review, the authors conducted a literature search in PubMed/MEDLINE

databases for experimental studies involving the most common methods of blood collection used in animal studies for PRF production.

### 3. Overview of Animal Research

Animal care is a crucial aspect of any animal study, as researchers are responsible for ensuring that their subjects are treated humanely and with the utmost respect. This consists of providing appropriate housing, nutrition, and care to minimize any potential harm that may be inflicted on them during the study. In addition, by establishing an ethical committee for animal studies, researchers can ensure that all aspects of the study are reviewed by an independent body to ensure compliance with ethical standards and regulations [28].

In addition to ethical oversight, guidelines are essential in any animal study. The ARRIVE guidelines were developed to provide a checklist of information that should be included in reports of *in vivo* experiments [29]. These guidelines help to ensure transparency and consistency in reporting by providing a standardized format that contains information about animal welfare, housing, and health status. These guidelines also ensure that researchers provide sufficient detail on their experimental design, methods, and results, allowing others to reproduce the study if necessary. They are of special interest in platelet concentrate research as these products depend on the content of several growth factors and inflammatory mediators whose production and release may be affected by different biological factors, health status, and stress.

Similarly, the PREPARE guidelines were developed to provide a framework for the planning phase of animal studies [30]. By considering all the factors that may influence the study's outcome during the planning phase, researchers can ensure their study is well-designed and scientifically rigorous. This includes considering factors such as the choice of animal model, sample size, experimental design, and statistical analysis.

The importance of these guidelines cannot be overstated, as they provide clear and concise instructions for the ethical use of animals in research. They promote transparency, consistency, and accountability, ultimately enhancing the research findings' credibility.

Sample size calculation is another critical aspect of studies involving animals [31,32]. By determining the minimum number of animals required to achieve statistical power, researchers can ensure that their research is adequately powered to detect any effects of the intervention or treatment being tested. Furthermore, sample size calculations consider factors such as the expected effect size, variability, and significance level to ensure the study is well-designed and scientifically rigorous. Adequate sample sizes also help to minimize the number of animals used in the study, reducing any potential harm that may be inflicted on them.

### 4. Considerations on Blood Collection

When collecting blood from animals for research, it is essential to prioritize the safety and well-being of the animals, which requires adhering to similar guidelines as those followed in human blood donation. One fundamental recommendation is the "10% rule", which states that the amount of blood drawn should not exceed 10% of the animal's total blood volume [33,34]. For instance, a 2 kg rabbit has an average blood volume of approximately 55–70 mL per kilogram of body weight, translating to a total blood volume of around 110–140 mL. Hence, based on the 10% rule, a maximum of 11–14 mL of blood can be safely collected from this rabbit to avoid any adverse effects. By following these guidelines, researchers can collect blood from animals safely and humanely in a manner that does not jeopardize their health.

Similarly, when collecting blood from rats, it is essential to use proper techniques and equipment. The amount of blood that can be safely collected from a rat depends on several factors, including body weight, age, and strain. As a general guideline, the amount of blood that can be safely drawn from a rat should not exceed 10% of its total blood volume. For example, a 250-g rat has a total blood volume of approximately 15–20 mL, so a maximum of 1.5–2 mL of blood can be safely collected from this rat.

In addition, ensuring safe and adequate blood collection involves adhering to proper techniques and equipment, and selecting the appropriate vessel and blood flow for the study type. The choice of vessel and blood flow depends on the desired blood by-product and its specific requirements. For instance, in PRP studies [35–39], anticoagulants are used, such as sodium citrate, which do not require fast blood flow to the collection tube. Conversely, for PRF studies, a vessel with rapid blood flow is necessary due to the absence of anticoagulants and the use of clot activators such as silica, making fast blood obtention essential to produce this blood concentrate.

### 5. Venipuncture of Rats

Blood collection in rats is a common procedure in many research studies that require blood samples for analysis (e.g., cytokines and growth factors) or for blood concentrate production [40–46]. There are several anatomical sites from where blood can be collected from rats; the most common are the tail vein, intracardiac function, and orbital sinus (Table 1).

**Table 1.** Data was collected from the PRF process using the rat model in the last five years, including one from 2023.

Author and Year	Breed	Animal Size	Number of Animals	Blood Collected (mL)	Centrifuge (RPM/min or RCF)	Donor Area
Akyildiz et al. (2018) [44]	Sprague-Dawley rats	340–380 g	23	4	3000 rpm/10 min	Intracardiac puncture
Alizadeh et al. (2023) [45]	Wistar rats	230–300 g	NR	NR	2700 rpm/12 min	Orbital sinus
Alsharif et al. (2020) [46]	Wistar rats	300–360 g	10 (PRF group)	4	2000 rpm/10 min (400 RCF)	Orbital sinus
Awadeen et al. (2020) [47]	Sprague-Dawley rats	NR	63	10	3000 rpm/10 min	Orbital sinus
da Silva et al. (2022) [11]	Wistar rats	350–450 g	24	3	2700 rpm/12 min (701 RCF-max) and 1500 rpm/14 min (216 RCF-max)	Intracardiac puncture
Demirel et al. (2018) [48]	Sprague-Dawley rats	400–450 g	28	16	3000 rpm/13 min	Intracardiac puncture
Engler-Pinto et al. (2019) [42]	Wistar rats	250–300 g	8	3.5	2700 rpm/12 min	Intracardiac puncture
Greco et al. (2019) [49]	Wistar rats	220–420 g	35	10	1300 rpm/8 min (400 RCF)	Intracardiac puncture
Greco et al. (2019) [50]	Wistar rats	220–420 g	35 (scarified to produce PRF)	10	1300 rpm/8 min (400 RCF)	Intracardiac puncture
Huang et al. (2020) [23]	Sprague-Dawley rats	180–220 g	24 (sacrificed to produce PRF)	5	400 g/10 min	NR
Jamalpour et al. (2022) [51]	Wistar rats	300–350 g	60	2	1500 rpm/14 min and 2700 rpm/12 min	Orbital sinus
Mirhaj et al. (2022) [52]	Wistar rats	250–300 g	3 (PRF group)	NR	2700 rpm/12 min	Orbital sinus
Mourad et al. (2022) [53]	Wistar rats	250–300 g	30	2	3000 rpm/10 min	Tail vein
Neves-Atti et al. (2022) [54]	Spontaneously hypertensive rats	250 g	40	6	3000 rpm/10 min	Intracardiac puncture
Nica et al. (2019) [55]	Wistar rats	460–550 g	40	9 to 12	450 g/12 min	Intracardiac puncture
Nugraha et al. (2018) [56]	Wistar rats	250 g	36	1.5	3000 rpm/10 min	Intracardiac puncture

Table 1. Cont.

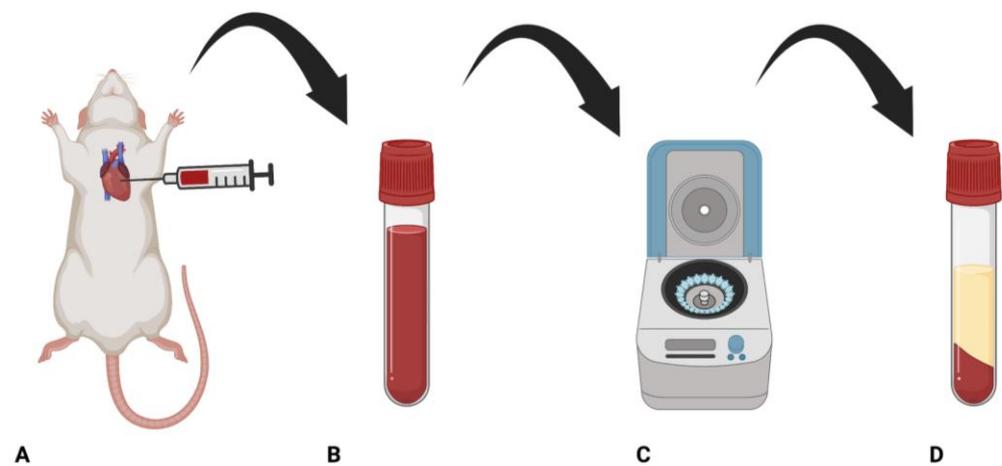
Author and Year	Breed	Animal Size	Number of Animals	Blood Collected (mL)	Centrifuge (RPM/min or RCF)	Donor Area
Özçay et al. (2018) [22]	Sprague-Dawley rats	250–300 g	40	1	3000 rpm/10 min	Intracardiac puncture
Özçay et al. (2020) [40]	Sprague-Dawley rats	250–300 g	10 (PRF group)	1	3000 rpm/10 min	Intracardiac puncture
Padilha et al. (2019) [57]	Wistar rats	300–400 g	1 animal scarified per group	9	3000 rpm/12 min	Intracardiac puncture
Rady et al. (2022) [19]	Wistar rats	175–200 g	36	2	3000 rpm/10 min	Tail vein
Silveira et al. (2022) [58]	Wistar rats	320 g	54 (2 animals scarified for PRF)	10	2700 rpm/12 min (857 RCF max)	Intracardiac puncture
Sumida et al. (2019) [59]	Wistar rats	400–450 g	23	6	890 g/13 min	Intracardiac puncture
Tavakoli et al. (2022) [60]	Wistar rats	400 g	NR	6	1500 rpm/14 min	Orbital sinus
Tayşi et al. (2018) [61]	Sprague-Dawley rats	240–260 g	60 (including a sacrification group)	10 to 15	3000 rpm/10 min (400 RCF)	Intracardiac puncture
Torul et al. (2018) [39]	Wistar rats	200–250 g	30	2	3000 rpm/10 min (400 RCF)	Tail vein
Vares et al. (2021) [62]	Wistar rats	250–280 g	32	2	3000 rpm/12 min (400 RCF)	Intracardiac puncture
Wang et al. (2021) [41]	Sprague-Dawley rats	NR	30	5	400 RCF/10 min	Abdominal aortic
Zhang et al. (2019) [63]	Sprague-Dawley rats	210–310 g	20	5	3000 rpm/10 min	Abdominal aortic
Zhang et al. (2022) [64]	Sprague-Dawley rats	250–300 g	32	3.5	600 RCF	Intracardiac puncture

RCF = relative centrifugal force; NR = not reported.

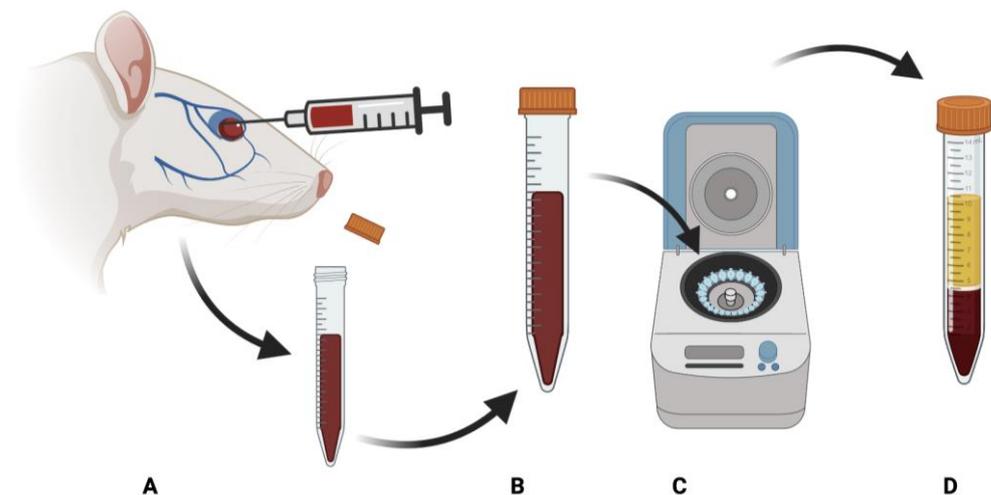
Blood collection from the tail vein is relatively easy and minimally invasive, making it a preferred option for many researchers. The rat's tail is first warmed with a heat lamp to dilate the veins, and sometimes a small incision is made to collect the blood. This method is used for collecting small amounts of blood and is suitable for tests that require minimal manipulation of the blood sample.

Intracardiac is a more invasive blood collection method involving accessing the heart directly. It requires surgical skills and experience and should be performed under anesthesia (Figure 1). This method allows for the collection of larger volumes of blood and is more suitable for tests that require more extensive manipulation of the blood sample. Therefore, intracardiac should be the first area of election to produce the PRF from the rats.

Table 1 shows another option for blood collection in rats, the orbital sinus that involves accessing the veins located behind the rat's eyes (Figure 2). Although this blood collection method is simple to carry out and is applicable for tests, with minimal manipulation of blood samples and smaller volumes, it may be ineffective in situations where low blood flow is an issue. Insufficient blood flow, which can result from certain medical conditions or injuries, may restrict the amount of blood obtained using this method. Thus, in such circumstances, alternative blood collection methods such as venous or arterial puncture may need to be used to obtain the required amount of blood for testing. These observations provide a comprehensive view of the advantages and limitations of this blood collection method and emphasize the significance of considering alternative techniques when blood flow is reduced.



**Figure 1.** (A) Intracardiac puncture in the rat's heart using a syringe with a needle ranging between 18 to 23G; (B) Blood placed in tube (without additives); (C) The tube is then quickly placed in the centrifuge, following protocols for PRF production, rpm, time, and relative centrifugal force (RCF); (D) PRF is produced after following the previous steps.



**Figure 2.** (A) Orbital sinus puncture in the rat's orbital area using a syringe with a needle ranging between 21–23G; (B) Blood placed in a tube/falcon (without additives); (C) The tube is then quickly placed in the centrifuge, following protocols for PRF production, rpm, time, and RCF; (D) PRF is produced after following the previous steps.

Once the blood sample is collected for PRF production, it is essential to understand that the coagulation time for rat blood is around 2–5 min, which is faster than the human coagulation time of 5–10 min [65–70]. Considering this information, the researcher needs a fast blood collection from a vessel with satisfactory blood flow. After that, the tube must go quickly to the centrifugation process.

In terms of the number of cells in the blood, rats have a higher number of red blood cells and a lower number of white blood cells compared to humans. It is important to note that the platelet count in rats can vary widely depending on various factors such as age, sex, strain or breed, and health status. The platelet count in rats can range between 600,000 and 1,500,000. Therefore, it is recommended to consider these factors when interpreting the platelet count in rats for PRF production. Additionally, the quantity of cytokines and growth factors in rat blood varies based on these [71–73]. Therefore, it is essential to consider these factors when conducting research using rat blood samples and comparing data from different studies in the literature.

### 6. Venipuncture of Rabbits

Rabbits are another animal model used to study different types of blood concentrates [74–79]. Considering the animal’s anatomy, there are several areas where blood can be collected from rabbits. The most common areas found in studies involving PRF include the ear vein, intracardiac, and jugular (Table 2).

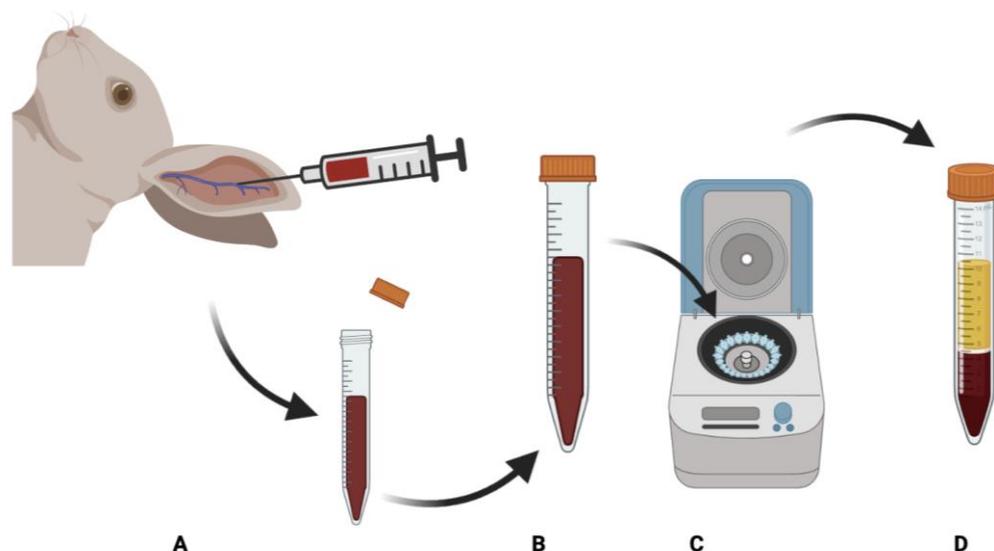
**Table 2.** Data was collected from the PRF process using the rabbit model in the last three years.

Author and Year	Breed	Animal Size	Number of Animals	Blood Collected (mL)	Centrifuge (RPM/min or RCF)	Donor Area
Choi et al. (2021) [77]	New Zealand	2.5–3 kg	33	5	2700 rpm/12 min	NR
Damayanti et al. (2022) [78]	New Zealand	3–4 kg	18	3	3200 rpm/10 min	Ear
Dereli-Can et al. (2020) [79]	New Zealand	3.0–3.5 kg	45	5	2700 rpm/12 min	Femoral vein
Karayürek et al. (2019) [80]	New Zealand	2.6–3.9 kg	28	8	3000 rpm/10 min	Ear
Kim et al. (2021) [81]	New Zealand	3.0–4.0 kg	12	10	3000 rpm/10 min	Ear
Kinoshita et al. (2021) [82]	New Zealand	3.5–4.2 kg	18	10	2400–3000 rpm/13 min	NR
Kızıldağ et al. (2020) [83]	New Zealand	3.0–3.5 kg	18	5	2700 rpm/12 min	Ear
Koyanagi et al. (2022) [74]	New Zealand	3.5–4.0 kg	5	2.5	700 RCF/12 min	Ear
Li et al. (2022) [73]	New Zealand	2 ± 0.2 kg	52	5	2000 RCF/5 min	Intracardiac puncture
Liu et al. (2021) [84]	New Zealand	3–4 kg	12	10	3000 rpm/10 min	Ear
Liu et al. (2019) [72]	New Zealand	2.8 and 4 kg	12	5	3000 rpm/10 min	Ear
Mogharehabed et al. (2022) [76]	New Zealand	1.5 kg	20	9	2700 rpm (408 RCF)/12 min	NR
Mu et al. (2020) [71]	New Zealand	3.0–3.5 kg	16	10	700 rpm/3 min	NR
Mudalal et al. (2019) [85]	New Zealand	3.0–3.5 kg	12	10	3000 rpm (1278 RCF)/12 min	NR
Rezuc et al. (2020) [86]	New Zealand	2 kg	12	8	3000 rpm/400 RCF/10 min	Ear
Salih et al. (2018) [87]	New Zealand	1.5–2 kg	20	3	3000 rpm/10 min	Intracardiac puncture
Şentürk et al. (2020) [14]	New Zealand	2.0–3.0 kg	27	10	3500 rpm/15 min	Ear
Shanei et al. (2022) [15]	New Zealand	2.5–3 kg	5	5	2700 rpm/8 min	Ear
Taufik et al. (2023) [16]	New Zealand	2.0–3.5 kg	15	10	3000 rpm/10 min	Ear
Wang et al. (2022) [17]	New Zealand	3–3.5 kg	10	10	3000 rpm/12 min	Ear
Wong et al. (2021) [88]	New Zealand	2–2.5 kg	24	8	2700 rpm/10 min	Ear
Zalama et al. (2022) [18]	New Zealand	N/D	30	NR	NR	NR
Zhang et al. (2023) [75]	New Zealand	3.5 ± 0.5 kg	9	5	1300 rpm/14 min	NR

RCF = relative centrifugal force; NR = not reported.

The ear vein is a less-invasive method for collecting small volumes of blood from rabbits (Figure 3). This method is relatively easy, making it a preferred option for many

researchers (Table 2). The rabbit's ear usually is warmed to dilate the veins to collect the blood. The ear vein is the most common area used by researchers to produce PRF (Table 2). However, this area does not offer a large amount of blood in a favorable flow to produce the PRF membrane. Therefore, it should not be considered the first area of election to produce more than one or a larger PRF membrane.



**Figure 3.** (A) Ear vein puncture in the rabbit using a syringe with a needle ranging between 21 to 23G; (B) Blood placed in a tube/falcon (without additives); (C) The tube is then quickly placed in the centrifuge, following protocols for PRF production, rpm, time, and RCF; (D) PRF is produced after following the previous steps.

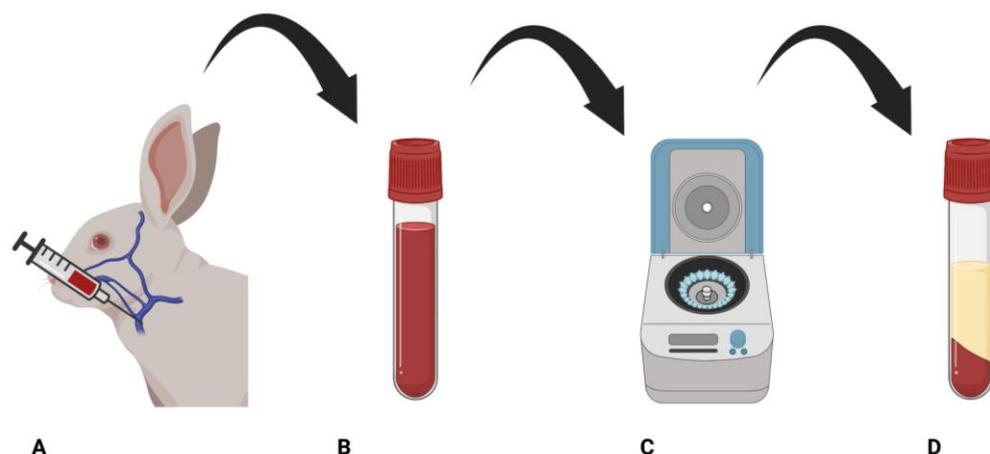
Intracardiac function is a more invasive method of blood collection that involves accessing the heart directly. It requires surgical skills and experience and should be performed under anesthesia. As was described for the rat model, this method allows for the collection of larger volumes of blood and is more suitable for extensive manipulation of the blood sample.

The jugular vein puncture is another method for collecting blood in rabbits that involves accessing the veins located in the neck area (Figure 4). This method is convenient to perform, and it is used for collecting larger volumes of blood and is best suited for the production of PRF and other blood concentrates. The jugular vein allows a faster and more significant amount of blood than the marginal ear vein, making it less stressful for the rabbit than a heart puncture [87].

The coagulation time for rabbit blood is around 5 to 10 min, which is similar to the coagulation time in humans. In addition, they are often considered the best animal model to study human platelets due to their similarities in platelet function and regulation [88–95]. Regarding platelet count, rabbits have a similar range of platelet numbers compared to humans, with an average platelet count ranging from 150,000 to 450,000 platelets per microliter of blood. However, there may be some differences in the size and distribution of platelets between rabbits and humans, which can impact platelet function and response to different stimuli [70,96].

In addition, comparing the number of cells in the blood, rabbits have a similar range of red and white blood cells compared to humans. And the number of cytokines and growth factors in rabbit blood can vary based on the age, sex, and strain of the rabbit [96,97]. Therefore, it is crucial to consider these factors when conducting research using rabbit blood samples [70].

Regarding coagulation time, rabbit blood has a slightly longer coagulation time than rat blood. However, both are faster than the human coagulation time.



**Figure 4.** (A) The jugular vein puncture in the rabbit’s neck using a syringe with a needle ranging between 18–23G; (B) Blood placed in a tube/falcon (without additives); (C) The tube is then quickly placed in the centrifuge, following protocols for PRF production, rpm, time, and RCF; (D) PRF is produced after following the previous steps.

### 7. Venipuncture of Dogs

Blood collection from dogs for experimental research involving blood concentrates is a common practice. The most commonly used methods for blood collection in dogs include venipuncture of the cephalic, saphenous (lower limb veins), or jugular veins (Table 3). The choice of the site for blood collection depends on the animal’s size, temperament, and the amount of blood needed for the study.

**Table 3.** Data was collected from the PRF process using the dog model.

Author and Year	Breed	Animal Size	Number of Animals	Blood Collected (mL)	Centrifuge (RPM/min or RCF)	Donor Area
Alenazy et al. (2021) [95]	Mixed breed dog	NR	4	10	3000 rpm/10 min	NR
Anwar et al. (2022) [96]	Mixed breed dog	9–14 kg	9	20	2500 rpm/15 min	NR
Benalcázar et al. (2022) [97]	Beagle dog	NR	13	18	2700 rpm/12 min	NR
El Halaby et al. (2020) [98]	Mixed breed dog	NR	9	20	3000 rpm/10 min	Right cephalic vein
Jeong et al. (2013) [99]	NR	10–15 kg	6	10	400 RCF/10 min	NR
Ji et al. (2015) [100]	NR	12–15 kg	NR	5	3000 rpm/5 min	Lower limb vein
Kazemi et al. (2014) [101]	Mixed breed dog	20–30 kg	12	20	3000 rpm/10 min	Jugular vein
Kazemi et al. (2017) [102]	Mixed breed dog	18–40 kg	12	20	3000 rpm/10 min	Jugular vein
Mohammed et al. (2021) [103]	Mixed breed dog	18–23 kg	8	5	400 RCF/10 min	Lower limb vein
Neiva et al. (2016) [104]	Beagle dog	NR	8	NR	2700 rpm/12 min	NR
Park et al. (2022) [105]	Beagle dog	NR	7	NR	408 RCF—time NR	NR
Park et al. (2016) [106]	NR	15 kg	6	10	3000 rpm/12 min	NR
Park et al. (2023) [107]	Beagle dog	15 kg	7	20	1300 rpm/8 min	Jugular vein
Xuan et al. (2014) [108]	Mixed breed dog	15–20 kg	6	20	2400 rpm/10 min	NR
Zhou et al. (2017) [109]	Beagle dog	NR	3	10	NR	Antecubital vein

RCF = relative centrifugal force; NR = not reported.

The coagulation time in dogs is similar to that of humans, usually taking around 2 to 10 min. The number of cells in the blood, cytokines, and growth factors in dogs is also similar to those found in humans [110,111]. However, it is important to note that there can be individual variations in these factors between dogs, as well as between different breeds and ages.

When compared to other animals used in experimental research, such as rats or rabbits, dogs have a longer coagulation time. This difference should be considered when designing studies that involve clotting factors or blood coagulation.

Regarding platelet count, dogs have a higher platelet count than humans, with an average of 200,000 to 500,000 platelets per microliter of blood [111]. Thus, this difference should also be taken into account when designing experiments that involve platelets.

### 8. Venipuncture of Pigs and Mini-Pigs

Blood collection in pigs and mini-pigs for studies in blood concentrates is a commonly used technique in experimental research [112–117]. The choice of blood collection site in pigs and mini-pigs will depend on the size and age of the animal. In PRF studies, the most commonly used areas for blood collection in pigs and mini-pigs are the precaval vein, jugular vein, and ear vein (Table 4).

**Table 4.** Data was collected from the PRF process using the pig model.

Author and Year	Breed	Animal Size	Number of Animals	Blood Collected (mL)	Centrifuge (RPM/min or RCF)	Donor Area
Chen et al. (2014) [113]	Mini-pig	25–30 kg	20	8	3000 rpm/10 min	Superior vena cava
Li et al. (2013) [116]	Pig	NR	NR	10	2100 rpm/12 min	Precaval vein
Li et al. (2014) [117]	Pig	NR	NR	10	2100 rpm/12 min	Precaval vein
Sheu et al. (2017) [118]	Mini-pig	21.8 kg	6	8	3000 rpm/10 min	Right jugular vein
Tsai et al. (2019) [114]	Mini-pig	26.6 ± 4.1 kg	6	40	1300 g/15 min	Internal jugular vein
Yang et al. (2012) [115]	Pig	6.8–11.2 kg	21	8	3000 rpm/10 min	Right jugular vein
Yilmaz et al. (2014) [112]	Pig	60 ± 5 kg	3	10	400 RCF/10 min	Ear vein

RCF = relative centrifugal force; NR = Not reported.

Overall, pigs and mini-pigs are valuable models for experimental research. The use of pigs and mini-pigs in biomedical research can provide important insights into human health and disease. Careful consideration should be given to the choice of blood collection site to ensure minimal stress to the animal and maximum accuracy of results.

The time for pig blood coagulation is approximately 2 to 5 min, which is similar to human coagulation. This time may vary depending on the breed, age, and health status of the animal. The number of cells in the blood, the quantity of cytokines, and the growth factors in pig blood are also similar to that in humans. However, the platelet range is between 250,000 to 600,000 per microliter of blood [67].

It is worth noting that pigs are often used as animal models for studying human health and diseases. This is due to their anatomical and physiological similarity to humans and their porcine genome being three times closer to that of humans than the rat’s genome [119]. However, researchers should be cautious when handling pigs and mini-pigs for PRF studies, as their coagulation times can be faster than that of humans.

### 9. Venipuncture of Goats

Blood collection from goats for experimental research involving blood concentrates is an important practice. The most common method for blood collection in goats is through the jugular vein.

The coagulation time in goats is longer than that of humans, usually taking around 3 to 7 min. The number of cells in the blood, cytokines, and growth factors in goats are also similar to those found in humans. However, similar to dogs, there can be individual variations in these factors between goats, as well as between different breeds and ages [67,110].

When comparing platelet counts, goats exhibit a range similar to that of humans. On average, goats have between 100,000 and 500,000 platelets per microliter of blood, while humans typically possess 150,000 to 450,000 platelets per microliter of blood. This difference should also be taken into account when designing experiments that involve platelets [67].

It is important to note that goats are particularly useful as models for human research in studying blood products, particularly fibrinogen, due to the similarity of goat and human fibrinogen structures.

The number of cells, cytokines, and growth factors in goats is similar to those found in humans, and goats are particularly useful in studying fibrinogen [120,121]. The lower platelet count in goats compared to humans should be considered when designing experiments that involve platelets.

To perform dependable experimental studies on platelet concentrates, researchers must consider several vital factors that can affect the results. These factors include each animal’s age, gender, strain or breed, and overall health, as they can significantly influence platelet count, coagulation time (Table 5), and their corresponding averages. By addressing these factors, studies can more effectively ensure accurate and consistent findings.

**Table 5.** Comparison of platelet count and coagulation time between humans and various animal models: an overview.

Animal	Platelet Count (Platelets/ $\mu$ L)	Coagulation Time (Min)
Human	150,000–450,000	5 to 10
Rat	600,000–1,500,000	2 to 5
Rabbit	150,000–450,000	5 to 10
Dog	200,000–500,000	2 to 10
Mini-pig	250,000–600,000	2 to 5
Goat	100,000–500,000	3 to 7

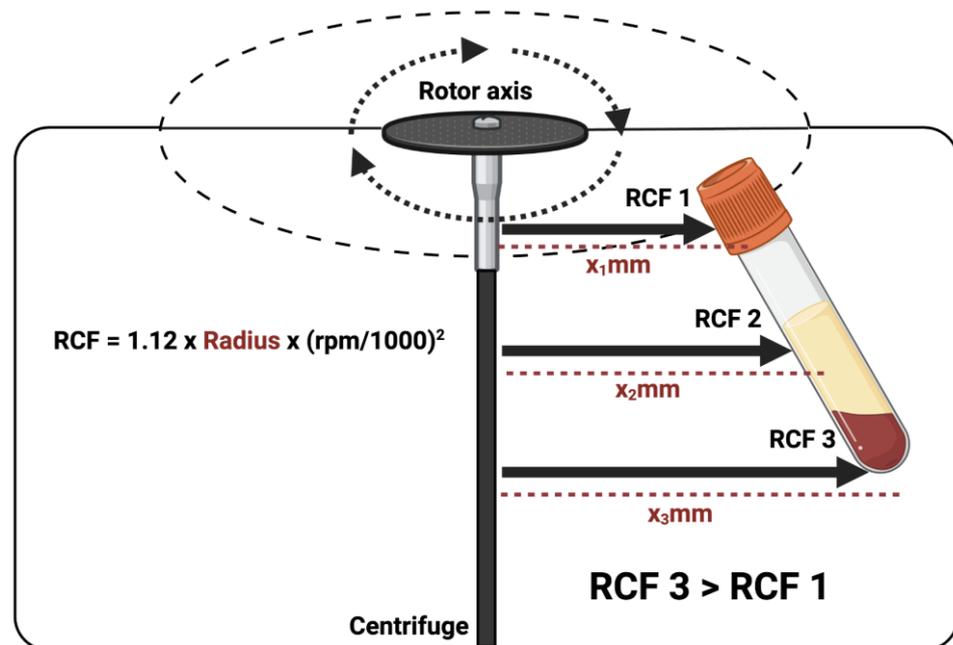
### 10. Considerations on the Relative Centrifugal Force

Producing blood concentrates, such as PRF, requires precise adherence to protocols; one of these is accurately calculating the relative centrifugal force (RCF) or g force required for the centrifugation process. Errors in RCF calculations can significantly affect the quality and reproducibility of the final product, making it vital to ensure accurate RCF calculations in every study. Standardizing RCF values across different studies can also enhance comparability and facilitate the transfer of scientific knowledge from animal to human studies.

The RCF value is determined by using a formula that considers the rotor’s radius and the revolutions per minute (rpm). The formula is an essential component of the blood concentrate production process, and any errors in calculating the RCF value can significantly impact the final product’s quality. Therefore, it is crucial to ensure that the formula is accurately applied in every study to produce reliable and reproducible results. In addition, it is recommended to use standardized methods for determining the RCF value to enhance comparability between different studies. Overall, correctly applying the RCF formula is critical for producing blood concentrates necessary for various scientific and clinical applications [122–126].

Communication of scientific and clinical protocols is crucial to ensure the reproducibility of human studies in a standard format. The principles of translational research determine that all details of a method of study should be reported to facilitate the transfer of scientific knowledge of animal studies to humans. However, inaccuracies in reports may impair the translation of research results. The PRF protocol is an example of such inaccuracy. The protocol reports the  $400\times g$  centrifugation RCF but does not specify the location of the rotor

in which the RCF was measured (mm). Therefore, to standardize the terminology for PRF production, it is necessary to establish an approach that focuses on the RCF obtained from the minimum radius ( $\times 1$  mm), average radius ( $\times 2$  mm), and maximum radius ( $\times 3$  mm), as illustrated in Figure 5. In doing so, animal studies can be conducted more accurately, and the results can be applied more reliably to human studies.



**Figure 5.** Schematic illustration of the blood centrifugation process, highlighting the minimum radius ( $\times 1$  mm), average radius ( $\times 2$  mm), and maximum radius ( $\times 3$  mm). It is important to note that the relative centrifugal force (RCF) calculation is higher for  $\times 3$  mm than  $\times 1$  mm. Additionally, for studies that use small tubes and low amounts of animal blood and for those using a commercial centrifuge to produce PRF, the authors should consider the average radius ( $\times 2$  mm) to provide the RCF in their methods.

Obtaining standardization in centrifugal calibration is crucial for reliable and reproducible results in the production of blood concentrate. The maximum RCF ( $\times 3$  mm) is the most commonly used parameter for centrifuge calibration, as it ensures the best standardization in different models and brands.

In order to have accurate and consistent results in PRF production, it is recommended to follow the same standardized standard for human studies and use the maximum radius ( $\times 3$  mm) to determine the RCF value. This value can be found in the literature [126] or obtained from the centrifuge manufacturer.

However, it is essential to note that the location of the  $\times 3$  mm may vary depending on the size and shape of the tube or hawk used for the study. Therefore, it is recommended to include a  $\times 2$  mm measurement as a standard in the study method. This measurement provides a reliable alternative to  $\times 3$  mm and can be used when the size or shape of the tube or Falcon prevents the use of  $\times 3$  mm.

In addition to the use of standard measurements, it is also crucial to report all the details of the centrifuge protocol in a standardized format to ensure reproducibility and comparability between studies. This includes reporting the rotor's location, the centrifuge's time and speed, and other relevant details. Following these guidelines, researchers can improve the accuracy and reliability of their results and promote the advancement of translational research in blood concentrate production.

It was important to identify the correct protocol for each centrifuge. The maximum RCF ( $\times 3$  mm) is commonly used for centrifugal calibration to obtain a more reliable standardization. Consequently, in the standardization published for human studies, the

maximum radius should be considered to determine the value of the RCF in the production of blood concentrate using several centrifuges available on the market. The  $\times 3$  mm information can be found in the literature or may be offered by the manufacturer.

The location of the  $\times 3$  mm mark for each study may also vary depending on the size of the tube or falcon used and the centrifuge radius. If the  $\times 3$  mm mark corresponds to a distance larger than the tube's size, the authors should consider adding a  $\times 2$  mm mark in the study's methods to standardize the RCF used in the protocol.

In order to ensure consistency and quality in PRF production, it is important to follow a standardized protocol. Protocol deviation can result in variations in the fibrin mesh, cell count, and growth factor content, making the scientific data obtained incomparable and potentially useless. Therefore, adhering to the established procedures for reliable and reproducible results is crucial.

## 11. Conclusions

Overall, the study concludes that standardization of animal models and techniques is essential for reducing the gap between laboratory research and clinical applications. It is crucial to report detailed information about the materials and vessel used for blood collection, the type of centrifuge used, rpm, RCF, and the maximum or average radius for the calculus, as well as the size of the tube and the amount of blood collected, to ensure reproducibility of results.

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## References

- Butler, D. Crossing the valley of death: A chasm has opened up between biomedical researchers and the patients who need their discoveries. Declan Butler asks how the ground shifted and whether the US National Institutes of Health can bridge the gap. *Nature* **2008**, *453*, 840–843. [[CrossRef](#)] [[PubMed](#)]
- Viergever, R.F.; Li, K. Trends in global clinical trial registration: An analysis of numbers of registered clinical trials in different parts of the world from 2004 to 2013. *BMJ Open* **2015**, *5*, e008932. [[CrossRef](#)] [[PubMed](#)]
- Collins, F.S.; Tabak, L.A. Policy: NIH plans to enhance reproducibility. *Nature* **2014**, *505*, 612–613. [[CrossRef](#)] [[PubMed](#)]
- Choukroun, J.; Adda, F.; Schoeffler, C.; Vervelle, A. Une opportunité en paro-implantologie: Le PRF. *Implantodontie* **2001**, *42*, e62.
- da Silva, M.T.; Mourão, C.F.d.A.B.; Mello-Machado, R.C.; Montemezzi, P.; Barbosa, R.d.L.; Sartoretto, S.C.; Leite, P.E.C.; Javid, K.; Kawase, T.; Alves, G.G. Effects of Leukocyte-Platelet-Rich Fibrin (L-PRF) on Pain, Soft Tissue Healing, Growth Factors, and Cytokines after Third Molar Extraction: A Randomized, Split-Mouth, Double-Blinded Clinical Trial. *Appl. Sci.* **2021**, *11*, 1666. [[CrossRef](#)]
- Mourão, C.F.d.A.B.; de Mello-Machado, R.C.; Javid, K.; Moraschini, V. The use of leukocyte-and platelet-rich fibrin in the management of soft tissue healing and pain in post-extraction sockets: A randomized clinical trial. *J. Cranio-Maxillofac. Surg.* **2020**, *48*, 452–457. [[CrossRef](#)]
- Mourão, C.F.d.A.B.; Valiense, H.; Melo, E.R.; Mourão, N.B.M.F.; Maia, M.D.-C. Obtention of injectable platelets rich-fibrin (i-PRF) and its polymerization with bone graft. *Rev. Colégio Bras. Cir.* **2015**, *42*, 421–423. [[CrossRef](#)]
- de Almeida Barros Mourao, C.F.; Lourenco, E.S.; Nascimento, J.R.B.; Machado, R.C.M.; Rossi, A.M.; Leite, P.E.C.; Granjeiro, J.M.; Alves, G.G.; Calasans-Maia, M.D. Does the association of blood-derived growth factors to nanostructured carbonated hydroxyapatite contributes to the maxillary sinus floor elevation? A randomized clinical trial. *Clin. Oral Investig.* **2019**, *23*, 369–379. [[CrossRef](#)]

9. Dohan, D.M.; Choukroun, J.; Diss, A.; Dohan, S.L.; Dohan, A.J.; Mouhyi, J.; Gogly, B. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part III: Leucocyte activation: A new feature for platelet concentrates? *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontol.* **2006**, *101*, e51–e55. [[CrossRef](#)]
10. Lourenco, E.S.; Mourao, C.; Leite, P.E.C.; Granjeiro, J.M.; Calasans-Maia, M.D.; Alves, G.G. The in vitro release of cytokines and growth factors from fibrin membranes produced through horizontal centrifugation. *J. Biomed. Mater. Res. Part A* **2018**, *106*, 1373–1380. [[CrossRef](#)]
11. da Silva, L.M.P.; Sávio, D.S.F.; de Ávila, F.C.; Vicente, R.M.; Reis, G.G.D.; Denardi, R.J.; da Costa, N.M.M.; Silva, P.H.F.; Mourão, C.; Miron, R.J.; et al. Comparison of the effects of platelet concentrates produced by high and low-speed centrifugation protocols on the healing of critical-size defects in rat calvaria: A microtomographic and histomorphometric study. *Platelets* **2022**, *33*, 1175–1184. [[CrossRef](#)] [[PubMed](#)]
12. Mourão, C.F.d.A.B.; Gheno, E.; Lourenço, E.S.; de Lima Barbosa, R.; Kurtzman, G.M.; Javid, K.; Mavropoulos, E.; Benedicenti, S.; Calasans-Maia, M.D.; de Mello Machado, R.C. Characterization of a new membrane from concentrated growth factors associated with denaturated Albumin (Alb-CGF) for clinical applications: A preliminary study. *Int. J. Growth Factors Stem Cells Dent.* **2018**, *1*, 64. [[CrossRef](#)]
13. Fujioka-Kobayashi, M.; Schaller, B.; Mourão, C.; Zhang, Y.; Sculean, A.; Miron, R.J. Biological characterization of an injectable platelet-rich fibrin mixture consisting of autologous albumin gel and liquid platelet-rich fibrin (Alb-PRF). *Platelets* **2020**, *31*, 74–81. [[CrossRef](#)]
14. Şentürk, F.; Bahadır, O.; Aktaş, O.; Bıyık, A.F.; Ercan, E. Effects of titanium prepared platelet rich fibrin on facial nerve regeneration: An experimental study. *Braz. J. Otorhinolaryngol.* **2022**, *88*, 867–874. [[CrossRef](#)]
15. Shanei, F.; Khoshzaban, A.; Taleghani, F.; Tehranchi, M.; Tayeed, M.H. The Effect of Low-Level Laser Therapy in Combination with Leukocyte- and Platelet- Rich Fibrin on Bone Regeneration in Rabbits' Calvarial Defects: Histologic and Histomorphometric Studies. *Cell J.* **2022**, *24*, 346–352. [[CrossRef](#)] [[PubMed](#)]
16. Taufik, S.A.; Dirja, B.T.; Utomo, D.N.; Usman, M.A.; Sakti, M.; Saleh, M.R.; Hatta, M.; Budu. Double membrane platelet-rich fibrin (PRF)—Synovium succeeds in regenerating cartilage defect at the knee: An experimental study on rabbit. *Heliyon* **2023**, *9*, e13139. [[CrossRef](#)]
17. Wang, J.; Sun, Y.; Liu, Y.; Yu, J.; Sun, X.; Wang, L.; Zhou, Y. Effects of platelet-rich fibrin on osteogenic differentiation of Schneiderian membrane derived mesenchymal stem cells and bone formation in maxillary sinus. *Cell Commun. Signal* **2022**, *20*, 88. [[CrossRef](#)]
18. Zalama, E.; Karrouf, G.; Rizk, A.; Salama, B.; Samy, A. Does zinc oxide nanoparticles potentiate the regenerative effect of platelet-rich fibrin in healing of critical bone defect in rabbits? *BMC Vet. Res.* **2022**, *18*, 130. [[CrossRef](#)] [[PubMed](#)]
19. Rady, D.; Mubarak, R.; Moneim, R.A.A. Healing capacity of bone marrow mesenchymal stem cells versus platelet-rich fibrin in tibial bone defects of albino rats: An in vivo study. *F1000Research* **2018**, *7*, 1573. [[CrossRef](#)]
20. Özçay, N.; Özdemir, H.; Besim, H. Role of platelet-rich fibrin on intestinal anastomosis wound healing in a rat. *Biomed. Mater.* **2018**, *13*, 045006. [[CrossRef](#)]
21. Huang, M.-L.; Zhai, Z.; Chen, Z.-X.; Yang, X.-N.; Qi, Z.-L. Platelet-rich fibrin membrane nerve guidance conduit: A potentially promising method for peripheral nerve injuries. *Chin. Med. J.* **2020**, *133*, 999–1001. [[PubMed](#)]
22. Dohan Ehrenfest, D.M.; Bielecki, T.; Jimbo, R.; Barbe, G.; Del Corso, M.; Inchingolo, F.; Sammartino, G. Do the fibrin architecture and leukocyte content influence the growth factor release of platelet concentrates? An evidence-based answer comparing a pure platelet-rich plasma (P-PRP) gel and a leukocyte-and platelet-rich fibrin (L-PRF). *Curr. Pharm. Biotechnol.* **2012**, *13*, 1145–1152. [[CrossRef](#)]
23. Cieslik-Bielecka, A.; Choukroun, J.; Odin, G.; Ehrenfest, M.D. L-PRP/L-PRF in esthetic plastic surgery, regenerative medicine of the skin and chronic wounds. *Curr. Pharm. Biotechnol.* **2012**, *13*, 1266–1277. [[PubMed](#)]
24. Andreone, A.; den Hollander, D. A retrospective study on the use of dermis micrografts in platelet-rich fibrin for the resurfacing of massive and chronic full-thickness burns. *Stem Cells Int.* **2019**, *2019*, 8636079. [[CrossRef](#)]
25. Yamakawa, S.; Hayashida, K. Advances in surgical applications of growth factors for wound healing. *Burn. Trauma* **2019**, *7*, 10. [[CrossRef](#)]
26. Weisel, J.W.; Litvinov, R.I. Fibrin Formation, Structure and Properties. *Subcell. Biochem.* **2017**, *82*, 405–456. [[CrossRef](#)]
27. Beristain-Covarrubias, N.; Perez-Toledo, M.; Thomas, M.R.; Henderson, I.R.; Watson, S.P.; Cunningham, A.F. Understanding Infection-Induced Thrombosis: Lessons Learned From Animal Models. *Front. Immunol.* **2019**, *10*, 2569. [[CrossRef](#)]
28. Hernandez, E.; Fawcett, A.; Brouwer, E.; Rau, J.; Turner, P.V. Speaking Up: Veterinary Ethical Responsibilities and Animal Welfare Issues in Everyday Practice. *Animals* **2018**, *8*, 15. [[CrossRef](#)] [[PubMed](#)]
29. Kilkenny, C.; Browne, W.J.; Cuthill, I.C.; Emerson, M.; Altman, D.G. Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. *J. Pharmacol. Pharmacother.* **2010**, *1*, 94–99. [[CrossRef](#)]
30. Smith, A.J.; Clutton, R.E.; Lilley, E.; Hansen, K.E.A.; Brattelid, T. PREPARE: Guidelines for planning animal research and testing. *Lab. Anim.* **2018**, *52*, 135–141. [[CrossRef](#)]
31. Charan, J.; Kantharia, N.D. How to calculate sample size in animal studies? *J. Pharmacol. Pharmacother.* **2013**, *4*, 303–306. [[CrossRef](#)]
32. Arifin, W.N.; Zahiruddin, W.M. Sample size calculation in animal studies using resource equation approach. *Malays. J. Med. Sci. MJMS* **2017**, *24*, 101. [[PubMed](#)]
33. Albus, U. *Guide for the Care and Use of Laboratory Animals*, 8th ed.; SAGE Publications Sage: London, UK, 2012.

34. Brouwer, G.J. Formulary for Laboratory Animals. *Lab. Anim.* **2005**, *39*, 457.
35. Ren, Z.Q.; Du, B.; Dong, H.J.; Duan, G.H.; Du, A.C.; Wang, Y.; Zhao, L.X.; Shao, W. Autologous Platelet-Rich Plasma Repairs Burn Wound and Reduces Burn Pain in Rats. *J. Burn. Care Res.* **2022**, *43*, 263–268. [[CrossRef](#)] [[PubMed](#)]
36. Stratakis, K.; Arkoumanis, T.; Liakea, A.; Nikiteas, N.; Zargaran, D.; Zargaran, A.; Kontzoglou, K.; Kyriakopoulou, P.; Perrea, D. Platelet-rich Plasma Gel versus Hyaluronic Acid on Prevention of Peritoneal Abdominal Adhesion Formation in Rats. *Chirurgia* **2022**, *117*, 585–593. [[CrossRef](#)] [[PubMed](#)]
37. Liao, C.H.; Lee, K.H.; Chung, S.D.; Chen, K.C.; Praveen Rajneesh, C.; Chen, B.H.; Cheng, J.H.; Lin, W.Y.; Chiang, H.S.; Wu, Y.N. Intracavernous Injection of Platelet-Rich Plasma Therapy Enhances Erectile Function and Decreases the Mortality Rate in Streptozotocin-Induced Diabetic Rats. *Int. J. Mol. Sci.* **2022**, *23*, 3017. [[CrossRef](#)]
38. Aboutaleb, H.; Alipour, F.; Ebrahimzadeh-Bideskan, A. The protective effect of co-administration of platelet-rich plasma (PRP) and pentoxifylline (PTX) on cyclophosphamide-induced premature ovarian failure in mature and immature rats. *Toxicol. Mech. Methods* **2022**, *32*, 588–596. [[CrossRef](#)]
39. Torul, D.; Bereket, M.C.; Onger, M.E.; Altun, G. Comparison of the regenerative effects of platelet-rich fibrin and plasma rich in growth factors on injured peripheral nerve: An experimental study. *J. Oral Maxillofac. Surg.* **2018**, *76*, 1823.e1821–1823.e1812. [[CrossRef](#)]
40. Ozcay, N.; Ozant, A.; Arslan, K.; Ozkayalar, H.; Besim, H. Platelet-rich fibrin can accelerate the healing of common bile duct anastomosis in a rat/Trombositten zengin fibrin sicanlarda safra yolu anastomoz yara iyilesmesini hizlandirabilir. *Turk. J. Surg.* **2020**, *36*, 256–264. [[CrossRef](#)]
41. Wang, Y.; Chen, X.; Mao, L.; Cui, L.; Bai, W. Therapeutic Effect of Platelet-Rich Fibrin Transplant on Formation of Thin Endometrium. *Exp. Clin. Transplant. Off. J. Middle East Soc. Organ Transplant.* **2021**, *19*, 600–608. [[CrossRef](#)] [[PubMed](#)]
42. Engler-Pinto, A.; Siessere, S.; Calefi, A.; Oliveira, L.; Ervolino, E.; de Souza, S.; Furlaneto, F.; Messori, M.R. Effects of leukocyte-and platelet-rich fibrin associated or not with bovine bone graft on the healing of bone defects in rats with osteoporosis induced by ovariectomy. *Clin. Oral Implant. Res.* **2019**, *30*, 962–976. [[CrossRef](#)] [[PubMed](#)]
43. Vickers, N.J. Animal communication: When i'm calling you, will you answer too? *Curr. Biol.* **2017**, *27*, R713–R715. [[CrossRef](#)] [[PubMed](#)]
44. Akyildiz, S.; Soluk-Tekkesin, M.; Keskin-Yalcin, B.; Unsal, G.; Yildiz, S.O.; Ozcan, I.; Cakarar, S. Acceleration of fracture healing in experimental model: Platelet-rich fibrin or hyaluronic acid? *J. Craniofacial Surg.* **2018**, *29*, 1794–1798. [[CrossRef](#)]
45. Alizadeh, M.; Salehi, S.; Tavakoli, M.; Mirhaj, M.; Varshosaz, J.; Kazemi, N.; Salehi, S.; Mehrjoo, M.; Abadi, S.A.M. PDGF and VEGF-releasing bi-layer wound dressing made of sodium tripolyphosphate crosslinked gelatin-sponge layer and a carrageenan nanofiber layer. *Int. J. Biol. Macromol.* **2023**, *223*, 123491. [[CrossRef](#)] [[PubMed](#)]
46. Alsharif, A.A.; Eltokhey, H.M.; Taiema, D.A. Platelet rich fibrin versus ozone gel for periodontal regeneration in induced rats' intrabony three-wall periodontal defects. *J. Oral Biol. Craniofacial Res.* **2020**, *10*, 639–649. [[CrossRef](#)] [[PubMed](#)]
47. Awadeen, M.A.; Al-Belasy, F.A.; Ameen, L.E.; Helal, M.E.; Grawish, M.E. Early therapeutic effect of platelet-rich fibrin combined with allogeneic bone marrow-derived stem cells on rats' critical-sized mandibular defects. *World J. Stem Cells* **2020**, *12*, 55. [[CrossRef](#)]
48. Demirel, E.; Yildiz, K.; Çadirci, K.; Aygün, H.; Şenocak, E.; Gündoğdu, B. Effect of platelet-rich fibrin on epidural fibrosis and comparison to ADCON<sup>®</sup> Gel and hyaluronic acid. *Acta Orthop. Et Traumatol. Turc.* **2018**, *52*, 469–474. [[CrossRef](#)]
49. Grecu, A.F.; Popa, D.G.; Lungulescu, C.V.; Ciucă, E.M.; Camen, A.; Marinescu, D.; Nica, O.; Busuioc, C.J.; Chen, F.I.; Ciurea, M.E. Histological findings from rat calvaria defect augmented with platelet-rich fibrin by using two consecutive periosteal incisions. *Rom. J. Morphol. Embryol. Rev. Roum. Morphol. Embryol.* **2019**, *60*, 111–118.
50. Grecu, A.F.; Grecu, D.; Nica, O.; Ciuca, E.M.; Camen, A.; Ciurea, M.E. A novel method of obtaining platelet rich fibrin from rats and quantifying platelet count. *Curr. Health Sci. J.* **2019**, *45*, 104.
51. Jamalpour, M.R.; Shahabi, S.; Baghestani, M.; Shokri, A.; Jamshidi, S.; Khazaei, S. Complementarity of surgical therapy, photobiomodulation, A-PRF and L-PRF for management of medication-related osteonecrosis of the jaw (MRONJ): An animal study. *BMC Oral. Health* **2022**, *22*, 241. [[CrossRef](#)]
52. Mirhaj, M.; Tavakoli, M.; Varshosaz, J.; Labbaf, S.; Jafarpour, F.; Ahmaditabar, P.; Salehi, S.; Kazemi, N. Platelet rich fibrin containing nanofibrous dressing for wound healing application: Fabrication, characterization and biological evaluations. *Biomater. Adv.* **2022**, *134*, 112541. [[CrossRef](#)]
53. Mourad, S.; Al-Dubai, S.; Elsayed, S.; El-Zehary, R. Efficacy of platelet-rich fibrin and tacrolimus on facial nerve regeneration: An animal study. *Int. J. Oral Maxillofac. Surg.* **2022**, *51*, 279–287. [[CrossRef](#)] [[PubMed](#)]
54. Atti, V.N.; Fernandes, M.; de Lima Figueiredo, G.S.; Roth, F.; Valente, S.G.; Nakachima, L.; Fernandes, C.; Dos Santos, J.G. Peripheral nerve regeneration in rats using nerve graft in a vein conduit pre-filled with platelet-rich fibrin (PRF). *Hand Surg. Rehabil.* **2023**, *42*, 61–68. [[CrossRef](#)] [[PubMed](#)]
55. Nica, O.; Popa, D.G.; Grecu, A.F.; Ciucă, E.M.; Ciurea, M.E. Effects of platelet rich fibrin on full thickness skin grafts in the rat model-planimetry results. *Curr. Health Sci. J.* **2019**, *45*, 278. [[PubMed](#)]
56. Nugraha, A.P.; Narmada, I.B.; Ernawati, D.S.; Dinaryanti, A.; Hendrianto, E.; Ihsan, I.S.; Riawan, W.; Rantam, F.A. Osteogenic potential of gingival stromal progenitor cells cultured in platelet rich fibrin is predicted by core-binding factor subunit- $\alpha 1$ /Sox9 expression ratio (in vitro). *F1000Research* **2018**, *7*, 1134. [[CrossRef](#)] [[PubMed](#)]

57. Motta Padilha, W.S.; Borges Soares, A.; Navarro-Junior, H.; César Joly, J.; Cristina Peruzzo, D.; Henrique Napimoga, M.; Ferreira Martinez, E. Histologic Evaluation of Leucocyte-and Platelet-Rich Fibrin in the Inflammatory Process and Repair of Noncritical Bone Defects in the Calvaria of Rats. *Int. J. Oral Maxillofac. Implant.* **2018**, *33*, 1206–1212. [[CrossRef](#)]
58. Silveira, B.B.B.; Teixeira, L.N.; Miron, R.J.; Martinez, E.F. Effect of platelet-rich fibrin (PRF) membranes on the healing of infected skin wounds. *Arch. Dermatol. Res.* **2022**, *315*, 559–567. [[CrossRef](#)]
59. Sumida, R.; Maeda, T.; Kawahara, I.; Yusa, J.; Kato, Y. Platelet-rich fibrin increases the osteoprotegerin/receptor activator of nuclear factor- $\kappa$ B ligand ratio in osteoblasts. *Exp. Ther. Med.* **2019**, *18*, 358–365. [[CrossRef](#)]
60. Tavakoli, M.; Mirhaj, M.; Salehi, S.; Varshosaz, J.; Labbaf, S.; Golshirazi, A.; Kazemi, N.; Haghighi, V. Coaxial electrospun angiogenic nanofiber wound dressing containing advanced platelet rich-fibrin. *Int. J. Biol. Macromol.* **2022**, *222*, 1605–1618. [[CrossRef](#)]
61. Tayşi, M.; Atalay, B.; Çankaya, B.; Yıldırım, S. Effects of single-and double-layered resorbable membranes and platelet-rich fibrin on bone healing. *Clin. Oral Investig.* **2018**, *22*, 1689–1695. [[CrossRef](#)]
62. Vares, P.; Dehghan, M.M.; Bastami, F.; Biazar, E.; Shamloo, N.; Keshel, S.H.; Khojasteh, A. Effects of platelet-rich fibrin/collagen membrane on sciatic nerve regeneration. *J. Craniofacial Surg.* **2021**, *32*, 794–798. [[CrossRef](#)] [[PubMed](#)]
63. Zhang, L.; Dong, Y.; Xue, Y.; Shi, J.; Zhang, X.; Liu, Y.; Midgley, A.C.; Wang, S. Multifunctional triple-layered composite scaffolds combining platelet-rich fibrin promote bone regeneration. *ACS Biomater. Sci. Eng.* **2019**, *5*, 6691–6702. [[CrossRef](#)] [[PubMed](#)]
64. Zhang, H.; Wang, S.; Lei, C.; Li, G.; Wang, B. Experimental study of negative pressure wound therapy combined with platelet-rich fibrin for bone-exposed wounds. *Regen. Med.* **2022**, *17*, 23–35. [[CrossRef](#)] [[PubMed](#)]
65. Lemini, C.; Jaimez, R.; Franco, Y. Gender and inter-species influence on coagulation tests of rats and mice. *Thromb. Res.* **2007**, *120*, 415–419. [[CrossRef](#)]
66. Lewis, J.H.; Van Thiel, D.H.; Hasiba, U.; Spero, J.A.; Gavalier, J. Comparative hematology and coagulation: Studies on rodentia (rats). *Comp. Biochem. Physiol. A Comp. Physiol.* **1985**, *82*, 211–215. [[CrossRef](#)] [[PubMed](#)]
67. Siller-Matula, J.M.; Plasenzotti, R.; Spiel, A.; Quehenberger, P.; Jilma, B. Interspecies differences in coagulation profile. *Thromb. Haemost.* **2008**, *100*, 397–404. [[CrossRef](#)]
68. Costa, N.O.; Forcato, S.; Cavichioli, A.M.; Pereira, M.R.F.; Gerardin, D.C.C. In utero and lactational exposure to triclocarban: Age-associated changes in reproductive parameters of male rat offspring. *Toxicol. Appl. Pharmacol.* **2020**, *401*, 115077. [[CrossRef](#)]
69. Somogyi, V.; Peto, K.; Deak, A.; Tanczos, B.; Nemeth, N. Effects of aging and gender on micro-rheology of blood in 3 to 18 months old male and female Wistar (CrL:WI) rats. *Biorheology* **2018**, *54*, 127–140. [[CrossRef](#)]
70. Pertsov, S.S.; Abramova, A.Y.; Chekhlov, V.V. Effect of Repeated Stress Exposures on the Blood Cytokine Profile in Rats with Different Behavioral Parameters. *Bull. Exp. Biol. Med.* **2022**, *172*, 397–401. [[CrossRef](#)]
71. Mu, Z.; He, Q.; Xin, L.; Li, Y.; Yuan, S.; Zou, H.; Shu, L.; Song, J.; Huang, Y.; Chen, T. Effects of injectable platelet rich fibrin on bone remodeling in combination with DBBM in maxillary sinus elevation: A randomized preclinical study. *Am. J. Transl. Res.* **2020**, *12*, 7312–7325.
72. Liu, Z.; Jin, H.; Xie, Q.; Jiang, Z.; Guo, S.; Li, Y.; Zhang, B. Controlled Release Strategies for the Combination of Fresh and Lyophilized Platelet-Rich Fibrin on Bone Tissue Regeneration. *BioMed Res. Int.* **2019**, *2019*, 4923767. [[CrossRef](#)] [[PubMed](#)]
73. Li, S.; Yang, H.; Duan, Q.; Bao, H.; Li, A.; Li, W.; Chen, J.; He, Y. A comparative study of the effects of platelet-rich fibrin, concentrated growth factor and platelet-poor plasma on the healing of tooth extraction sockets in rabbits. *BMC Oral. Health* **2022**, *22*, 87. [[CrossRef](#)] [[PubMed](#)]
74. Koyanagi, M.; Fujioka-Kobayashi, M.; Yoneyama, Y.; Inada, R.; Satomi, T. Regenerative Potential of Solid Bone Marrow Aspirate Concentrate Compared with Platelet-Rich Fibrin. *Tissue Eng. Part A* **2022**, *28*, 749–759. [[CrossRef](#)] [[PubMed](#)]
75. Zhang, Y.; Cao, C.; Li, J.; Liu, C.; Mi, K.; Zhang, X. Platelet-rich fibrin combined with new bone graft material for mandibular defect repair: A in vivo study on rabbits. *Dent. Mater. J.* **2023**, *42*, 241–247. [[CrossRef](#)]
76. Mogharehabed, A.; Torabinia, N.; Sharifi Darani, S.; Afshari, Z.; Yaghini, J. Effect of leukocyte and platelet-rich fibrin on free gingival graft healing: A clinical and histological study in rabbits. *J. Adv. Periodontol. Implant. Dent.* **2022**, *14*, 89–96. [[CrossRef](#)] [[PubMed](#)]
77. Choi, W.H.; Kim, Y.D.; Song, J.M.; Shin, S.H. Comparative study of bone regeneration using fibrin sealant with xenograft in rabbit sinus: Pilot study. *Maxillofac. Plast. Reconstr. Surg.* **2021**, *43*, 5. [[CrossRef](#)] [[PubMed](#)]
78. Damayanti, M.M.; Rachmawati, M. Pre-Clinical Study: Immunohistochemical evaluation of matrix metalloproteinase-13 on rabbit (*Oryctolagus cuniculus*) socket healing after application of platelet-rich fibrin with and without hydroxyapatite. *F1000Research* **2022**, *11*, 29. [[CrossRef](#)]
79. Dereli Can, G.; Akcan, G.; Can, M.E.; Akdere, Ö.E.; Çaylı, S.; Şimşek, G.; Gümüşderelioğlu, M. Surgical and Immunohistochemical Outcomes of Scleral Reconstruction with Autogenic, Allogenic and Xenogenic Grafts: An Experimental Rabbit Model. *Curr. Eye Res.* **2020**, *45*, 1572–1582. [[CrossRef](#)]
80. Karayürek, F.; Kadiroğlu, E.T.; Nergiz, Y.; Coşkun Akçay, N.; Tunik, S.; Ersöz Kanay, B.; Uysal, E. Combining platelet rich fibrin with different bone graft materials: An experimental study on the histopathological and immunohistochemical aspects of bone healing. *J. Cranio-Maxillo-Facial Surg. Off. Publ. Eur. Assoc. Cranio-Maxillo-Facial Surg.* **2019**, *47*, 815–825. [[CrossRef](#)]
81. Kim, B.J.; Kim, S.K.; Lee, J.H. Bone regeneration of demineralized dentin matrix with platelet-rich fibrin and recombinant human bone morphogenetic protein-2 on the bone defects in rabbit calvaria. *Maxillofac. Plast. Reconstr. Surg.* **2021**, *43*, 34. [[CrossRef](#)]

82. Kinoshita, T.; Hashimoto, Y.; Orita, K.; Nishida, Y.; Nishino, K.; Nakamura, H. Autologous Platelet-Rich Fibrin Membrane to Augment Healing of Microfracture Has Better Macroscopic and Histologic Grades Compared with Microfracture Alone on Chondral Defects in a Rabbit Model. *Arthrosc. J. Arthrosc. Relat. Surg. Off. Publ. Arthrosc. Assoc. N. Am. Int. Arthrosc. Assoc.* **2022**, *38*, 417–426. [[CrossRef](#)]
83. Kızıldağ, A.; Tasdemir, U.; Arabacı, T.; Kızıldağ, C.A.; Albayrak, M.; Şahin, B. Effects of Autogenous Tooth Bone Graft and Platelet-Rich Fibrin in Peri-Implant Defects: An Experimental Study in an Animal Model. *J. Oral Implantol.* **2020**, *46*, 221–226. [[CrossRef](#)]
84. Liu, F.; Zhao, Y. Effect of Co-transplanting Stromal Vascular Fraction-Gelatin and Platelet-Rich Fibrin on the Long-Term Maintenance of Fat Volume. *Aesthetic. Plast. Surg.* **2021**, *45*, 1853–1859. [[CrossRef](#)]
85. Mudalal, M.; Sun, X.; Li, X.; Zhou, Y. The evaluation of leukocyte-platelet rich fibrin as an anti-inflammatory autologous biological additive. A novel in vitro study. *Saudi Med. J.* **2019**, *40*, 657–668. [[CrossRef](#)] [[PubMed](#)]
86. Rezuc, A.; Saavedra, C.; Maass, R.; Poblete, C.; Nappe, C. Histological comparison of DBBM and platelet rich fibrin for guided bone regeneration in a rabbit model. *J. Oral Biol. Craniofacial Res.* **2020**, *10*, 287–293. [[CrossRef](#)] [[PubMed](#)]
87. Salih, S.I.; Al-Falahi, N.H.; Saliem, A.H.; Abedsalih, A.N. Effectiveness of platelet-rich fibrin matrix treated with silver nanoparticles in fracture healing in rabbit model. *Vet. World* **2018**, *11*, 944–952. [[CrossRef](#)] [[PubMed](#)]
88. Wong, C.C.; Yeh, Y.Y.; Chen, C.H.; Manga, Y.B.; Jheng, P.R.; Lu, C.X.; Chuang, E.Y. Effectiveness of treating segmental bone defects with a synergistic co-delivery approach with platelet-rich fibrin and tricalcium phosphate. *Mater. Sci. Engineering. C Mater. Biol. Appl.* **2021**, *129*, 112364. [[CrossRef](#)] [[PubMed](#)]
89. Abade dos Santos, F.A.; Carvalho, C.L.; Peleteiro, M.C.; Gabriel, S.I.; Patrício, R.; Carvalho, J.; Cunha, M.V.; Duarte, M.D. Blood collection from the external jugular vein of *Oryctolagus cuniculus algirus* sedated with midazolam: Live sampling of a subspecies at risk. *Wildl. Biol.* **2019**, *2019*, 1–10. [[CrossRef](#)]
90. Cooper, T.K.; Meyerholz, D.K.; Beck, A.P.; Delaney, M.A.; Piersigilli, A.; Southard, T.L.; Brayton, C.F. relevant conditions and pathology of laboratory mice, rats, gerbils, Guinea pigs, hamsters, naked mole rats, and rabbits. *ILAR J.* **2021**, *62*, 77–132. [[CrossRef](#)] [[PubMed](#)]
91. Parasuraman, S. Care and Handling of Laboratory Animals. In *Introduction to Basics of Pharmacology and Toxicology: Experimental Pharmacology: Research Methodology and Biostatistics*; Springer: Berlin, Germany, 2022; Volume 3, pp. 37–43.
92. Grgurevic, L.; Oppermann, H.; Pecin, M.; Erjavec, I.; Capak, H.; Pauk, M.; Karlovic, S.; Kufner, V.; Lipar, M.; Bubic Spoljar, J. Recombinant human bone morphogenetic protein 6 delivered within autologous blood coagulum restores critical size segmental defects of ulna in rabbits. *JBMR Plus* **2019**, *3*, e10085. [[CrossRef](#)]
93. Handtke, S.; Thiele, T. Large and small platelets—(When) do they differ? *J. Thromb. Haemost.* **2020**, *18*, 1256–1267. [[CrossRef](#)] [[PubMed](#)]
94. Kinlough-Rathbone, R.; Mustard, J.; Perry, D.; Dejana, E.; Cazenave, J.; Packham, M.; Harfenist, E. Factors influencing the deaggregation of human and rabbit platelets. *Thromb. Haemost.* **1983**, *49*, 162–167. [[CrossRef](#)] [[PubMed](#)]
95. Alenazy, M.S.; Al-Nazhan, S.; Mosadomi, H.A. Histologic, radiographic, and micro-computed tomography evaluation of experimentally enlarged root apices in dog teeth with apical periodontitis after regenerative treatment. *Curr. Ther. Res.* **2021**, *94*, 100620. [[CrossRef](#)]
96. Anwar, S.K.; Hamid, H.M.A. Immuno-histopathologic evaluation of mineralized plasmatic matrix in the management of horizontal ridge defects in a canine model (a split-mouth comparative study). *Odontology* **2022**, *110*, 523–534. [[CrossRef](#)]
97. Benalcazar Jalkh, E.B.; Tovar, N.; Arbex, L.; Kurgansky, G.; Torroni, A.; Gil, L.F.; Wall, B.; Kohanbash, K.; Bonfante, E.A.; Coelho, P.G. Effect of leukocyte-platelet-rich fibrin in bone healing around dental implants placed in conventional and wide osteotomy sites: A pre-clinical study. *J. Biomed. Mater. Res. Part B Appl. Biomater.* **2022**, *110*, 2705–2713. [[CrossRef](#)]
98. El Halaby, H.M.; Abu-Seida, A.M.; Fawzy, M.I.; Farid, M.H.; Bastawy, H.A. Evaluation of the regenerative potential of dentin conditioning and naturally derived scaffold for necrotic immature permanent teeth in a dog model. *Int. J. Exp. Pathol.* **2020**, *101*, 264–276. [[CrossRef](#)] [[PubMed](#)]
99. Jeong, K.-I.; Kim, S.-G.; Oh, J.-S.; Lee, S.-Y.; Cho, Y.-S.; Yang, S.-S.; Park, S.-C.; You, J.-S.; Lim, S.-C.; Jeong, M. Effect of platelet-rich plasma and platelet-rich fibrin on peri-implant bone defects in dogs. *J. Biomed. Nanotechnol.* **2013**, *9*, 535–537. [[CrossRef](#)]
100. Ji, B.; Sheng, L.; Chen, G.; Guo, S.; Xie, L.; Yang, B.; Guo, W.; Tian, W. The combination use of platelet-rich fibrin and treated dentin matrix for tooth root regeneration by cell homing. *Tissue Eng. Part A* **2015**, *21*, 26–34. [[CrossRef](#)]
101. Kazemi, D.; Fakhrjou, A.; Mirzazadeh Dizaji, V.; Khanzadeh Alishahi, M. Effect of autologous platelet rich fibrin on the healing of experimental articular cartilage defects of the knee in an animal model. *BioMed Res. Int.* **2014**, *2014*, 486436. [[CrossRef](#)]
102. Kazemi, D.; Asenjan, K.S.; Dehdilani, N.; Parsa, H. Canine articular cartilage regeneration using mesenchymal stem cells seeded on platelet rich fibrin: Macroscopic and histological assessments. *Bone Jt. Res.* **2017**, *6*, 98–107. [[CrossRef](#)]
103. Mohammed, A.A.; Elsherbini, A.M.; Ibrahim, F.M.; El-Meadawy, S.M.; Youssef, J.M. Biological effect of the nanocrystalline calcium sulfate bone graft in the periodontal regeneration. *J. Oral Biol. Craniofacial Res.* **2021**, *11*, 47–52. [[CrossRef](#)]
104. Neiva, R.F.; Gil, L.F.; Tovar, N.; Janal, M.N.; Marao, H.F.; Bonfante, E.A.; Pinto, N.; Coelho, P.G. The synergistic effect of leukocyte platelet-rich fibrin and micrometer/nanometer surface texturing on bone healing around immediately placed implants: An experimental study in dogs. *BioMed Res. Int.* **2016**, *2016*, 9507342. [[CrossRef](#)]

105. Park, G.; Jalkh, E.B.B.; Boczar, D.; Bergamo, E.T.; Kim, H.; Kurgansky, G.; Torroni, A.; Gil, L.F.; Bonfante, E.A.; Coelho, P.G. Bone regeneration at extraction sockets filled with leukocyte-platelet-rich fibrin: An experimental pre-clinical study. *Med. Oral Patol. Oral Y Cirugía Bucal* **2022**, *27*, e468. [[CrossRef](#)] [[PubMed](#)]
106. Park, H.-C.; Kim, S.-G.; Oh, J.-S.; You, J.-S.; Kim, J.-S.; Lim, S.-C.; Jeong, M.; Kim, J.-S.; Jung, C.; Kwon, Y.-S. Early bone formation at a femur defect using CGF and PRF grafts in adult dogs: A comparative study. *Implant. Dent.* **2016**, *25*, 387–393. [[CrossRef](#)] [[PubMed](#)]
107. Park, J.Y.; Hong, K.J.; Ko, K.A.; Cha, J.K.; Gruber, R.; Lee, J.S. Platelet-rich fibrin combined with a particulate bone substitute versus guided bone regeneration in the damaged extraction socket: An in vivo study. *J. Clin. Periodontol.* **2023**, *50*, 358–367. [[CrossRef](#)] [[PubMed](#)]
108. Xuan, F.; Lee, C.-U.; Son, J.-S.; Jeong, S.-M.; Choi, B.-H. A comparative study of the regenerative effect of sinus bone grafting with platelet-rich fibrin-mixed Bio-Oss<sup>®</sup> and commercial fibrin-mixed Bio-Oss<sup>®</sup>: An experimental study. *J. Cranio-Maxillofac. Surg.* **2014**, *42*, e47–e50. [[CrossRef](#)]
109. Zhou, R.; Wang, Y.; Chen, Y.; Chen, S.; Lyu, H.; Cai, Z.; Huang, X. Radiographic, histologic, and biomechanical evaluation of combined application of platelet-rich fibrin with blood clot in regenerative endodontics. *J. Endod.* **2017**, *43*, 2034–2040. [[CrossRef](#)]
110. Cortese, L.; Christopherson, P.W.; Pelagalli, A. Platelet function and therapeutic applications in dogs: Current status and future prospects. *Animals* **2020**, *10*, 201. [[CrossRef](#)] [[PubMed](#)]
111. Creevy, K.E.; Akey, J.M.; Kaeberlein, M.; Promislow, D.E. An open science study of ageing in companion dogs. *Nature* **2022**, *602*, 51–57. [[CrossRef](#)]
112. Yilmaz, D.; Dogan, N.; Ozkan, A.; Sencimen, M.; Ora, B.E.; Mutlu, I. Effect of platelet rich fibrin and beta tricalcium phosphate on bone healing. A histological study in pigs. *Acta Cir. Bras.* **2014**, *29*, 59–65. [[CrossRef](#)]
113. Chen, Y.; Niu, Z.; Xue, Y.; Yuan, F.; Fu, Y.; Bai, N. Improvement in the repair of defects in maxillofacial soft tissue in irradiated minipigs by a mixture of adipose-derived stem cells and platelet-rich fibrin. *Br. J. Oral Maxillofac. Surg.* **2014**, *52*, 740–745. [[CrossRef](#)] [[PubMed](#)]
114. Tsai, H.-C.; Chang, G.R.-L.; Fan, H.-C.; Ou-Yang, H.; Huang, L.-C.; Wu, S.-C.; Chen, C.-M. A mini-pig model for evaluating the efficacy of autologous platelet patches on induced acute full thickness wound healing. *BMC Vet. Res.* **2019**, *15*, 1–13. [[CrossRef](#)] [[PubMed](#)]
115. Yang, K.C.; Wang, C.H.; Chang, H.H.; Chan, W.P.; Chi, C.H.; Kuo, T.F. Fibrin glue mixed with platelet-rich fibrin as a scaffold seeded with dental bud cells for tooth regeneration. *J. Tissue Eng. Regen. Med.* **2012**, *6*, 777–785. [[CrossRef](#)] [[PubMed](#)]
116. Li, Q.; Pan, S.; Dangaria, S.J.; Gopinathan, G.; Kolokythas, A.; Chu, S.; Geng, Y.; Zhou, Y.; Luan, X. Platelet-rich fibrin promotes periodontal regeneration and enhances alveolar bone augmentation. *BioMed Res. Int.* **2013**, *2013*, 638043. [[CrossRef](#)] [[PubMed](#)]
117. Li, Q.; Reed, D.A.; Min, L.; Gopinathan, G.; Li, S.; Dangaria, S.J.; Li, L.; Geng, Y.; Galang, M.-T.; Gajendrareddy, P. Lyophilized platelet-rich fibrin (PRF) promotes craniofacial bone regeneration through Runx2. *Int. J. Mol. Sci.* **2014**, *15*, 8509–8525. [[CrossRef](#)] [[PubMed](#)]
118. Sheu, S.; Wang, C.; Pao, Y.; Fu, Y.; Liu, C.; Yao, C.; Kuo, T. The effect of platelet-rich fibrin on autologous osteochondral transplantation: An in vivo porcine model. *Knee* **2017**, *24*, 1392–1401. [[CrossRef](#)]
119. Walters, E.M.; Prather, R.S. Advancing swine models for human health and diseases. *Mo. Med.* **2013**, *110*, 212–215.
120. Marchelak, A.; Kolodziejczyk-Czepas, J.; Wasielewska, P.; Nowak, P.; Olszewska, M.A. The effects of *Prunus spinosa* L. flower extracts, model polyphenols and phenolic metabolites on oxidative/nitrative modifications of human plasma components with particular emphasis on fibrinogen in vitro. *Antioxidants* **2021**, *10*, 581. [[CrossRef](#)]
121. Dre'Von, A.D.; Holle, L.A.; Lin, F.-C.; Huffman, J.E.; Luyendyk, J.P.; Flick, M.J.; Smith, N.L.; de Vries, P.S.; Morrison, A.C.; Wolberg, A.S. Novel Genetic Regulators of Fibrinogen Synthesis Identified by an In Vitro Experimental Platform. *J. Thromb. Haemost.* **2022**, *21*, 522–533.
122. Al-Maawi, S.; Dohle, E.; Kretschmer, W.; Rutkowski, J.; Sader, R.; Ghanaati, S. A Standardized g-Force Allows the Preparation of Similar Platelet-Rich Fibrin Qualities Regardless of Rotor Angle. *Tissue Eng. Part A* **2022**, *28*, 353–365. [[CrossRef](#)]
123. Miron, R.J.; Xu, H.; Chai, J.; Wang, J.; Zheng, S.; Feng, M.; Zhang, X.; Wei, Y.; Chen, Y.; Mourão, C.F.d.A.B. Comparison of platelet-rich fibrin (PRF) produced using 3 commercially available centrifuges at both high (~700 g) and low (~200 g) relative centrifugation forces. *Clin. Oral Investig.* **2020**, *24*, 1171–1182. [[CrossRef](#)] [[PubMed](#)]
124. Castro Ana, B.; Catherine, A.; Xin, L.; Nelson, P.; Wim, T.; Marc, Q. Impact of g force and timing on the characteristics of PRF matrices. *Sci. Rep.* **2021**, *11*, 6038. [[CrossRef](#)] [[PubMed](#)]
125. Ghanaati, S.; Mourão, C.F.; Adam, E.H.; Sader, R.; Zadeh, H.H.; Al-Maawi, S. The role of centrifugation process in the preparation of therapeutic blood concentrates: Standardization of the protocols to improve reproducibility. *Int. J. Growth Factors Stem Cells Dent.* **2019**, *2*, 41–44. [[CrossRef](#)]
126. Miron, R.J.; Pinto, N.R.; Quirynen, M.; Ghanaati, S. *Standardization of Relative Centrifugal Forces in Studies Related to Platelet-Rich Fibrin*; Wiley Online Library: New York, NY, USA, 2019; Volume 90, pp. 817–820.

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