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RADIOGRAPHICAL AND BIOMECHANICAL EVALUATION OF AUTOLOGOUS PLATELET AND FIBRIN PLASMA DERIVATIVES IN BONE REGENERATION USING A RABBIT MODEL

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ABSTRACT

Bone defects resulting from trauma, infection, or complications of surgery remain a significant clinical challenge, particularly in cases where healing is delayed or incomplete. Regenerative medicine has introduced Platelet derivatives such as Platelet-rich Plasma (PRP) and Platelet-rich Fibrin (PRF) as adjuncts to accelerate bone repair by harnessing growth factors and biological scaffolds. This study investigated the radiographical and biomechanical effects of PRP, PRF, and their combination on the healing of critical-sized ulna defects in rabbits. Twenty-eight adult New Zealand White rabbits were randomly allocated into four groups: PRP-treated, PRF-treated, PRP+PRF combination, and untreated controls. We conducted standardized radiographic assessments at weeks 0, 4, 8, and 12, and evaluated biomechanical strength using Vickers hardness testing at week 12. Radiographs demonstrated progressive callus formation and defect bridging across all treated groups, with the PRF and PRP+PRF groups showing earlier and more consistent cortical remodeling than PRP or controls. Biomechanical analysis showed significantly higher hardness values in the PRF (145.5 ± 2.43 HV) and PRP+PRF (158.2 ± 5.95 HV) groups than in PRP (129.3 ± 5.47 HV) and controls (118.3 ± 1.53 HV), with the PRP+PRF group achieving the greatest mineralization ($P<0.001$). These findings highlight the superior role of PRF, either alone or combined with PRP in enhancing bone regeneration and fracture healing. In conclusion, both radiographic and biomechanical assessments suggest that PRF, particularly when combined with PRP, offers a more effective strategy for enhancing bone regeneration in orthopedic applications. The early advantage demonstrated by PRF, driven by its superior and sustained release of bioactive factors, provides compelling evidence of its potency during the critical early stages of healing.

Keywords: Bone regeneration, Platelet-rich plasma (PRP), Platelet-rich fibrin (PRF), Critical-sized bone defect, Biomechanical strength.

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1. INTRODUCTION

Musculoskeletal conditions remain a leading cause of pain, disability, and poor quality of life worldwide, placing a substantial burden on healthcare systems (Cieza et al., 2020; Liu et al., 2022b; Yusuf et al., 2026). Among these, bone fractures and defects pose a particular challenge, with a significant number experiencing delayed healing or non-union despite advancements in surgical techniques and implant technologies (Bell et al., 2016; Bowers & Anderson, 2024). This delay significantly increases healthcare costs because of prolonged hospital stays, repeat surgeries, and extended rehabilitation (Lidgren, 2008; Bell et al., 2016). Bone healing is a highly coordinated process involving three crucial phases: inflammation, repair (hard callus formation), and remodeling (Marsell & Einhorn, 2011; Einhorn & Clement, 2012; Abubakar et al., 2025b). Each stage depends on a complex interplay of signaling molecules, cytokines, and growth factors. Early inflammatory mediators such as Interleukin-1 (IL-1), Tumor Necrosis Factor-alpha (TNF-α), and Interleukin-6 (IL-6) have been shown to play vital roles in initiating the cascade of bone

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regeneration (Pape et al., 2010; Kaiser et al., 2018; Abubakar et al., 2025a; Sun et al., 2026). These cytokines are involved in controlling immune cell recruitment, as well as the stimulation of the differentiation of osteoblasts and mesenchymal stem cells (Lee et al., 2010; Oryan et al., 2017).

Advances in regenerative medicine have introduced Platelet-derived biomaterials, such as Platelet-rich Plasma (PRP) and Platelet-rich Fibrin (PRF), as novel adjuncts to hasten bone healing (Goswami et al., 2024). PRP has been reported to be rich in platelets and growth factors, including Platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), and insulin-like growth factor-1 (IGF-1), which are effective in augmenting angiogenesis, osteoblastic proliferation, and matrix formation (Foster et al., 2009; Liu et al., 2022a; Verma et al., 2023; Gharpinde et al., 2024; Odložilová et al., 2024). Upon activation, PRP releases a burst of cytokines and growth factors that modulate the inflammatory phase and stimulate bone tissue repair (Alsousou et al., 2009; Penteado et al., 2013; Pretorius et al., 2023; Lian et al., 2024; Khandan-Nasab et al., 2026).

Whereas PRP is primarily known for its rapid release of growth factors, PRF provides a fibrin matrix that supports sustained release and acts as a scaffold for cellular migration and attachment (Dohan Ehrenfest et al., 2009; Miron et al., 2024; Jia et al., 2024; Chou et al., 2025). This slow degradation of PRF supports long-term tissue regeneration and allows for the recruitment of progenitor cells to the injury site. Unlike PRP, PRF preparation does not require anticoagulants, thereby simplifying its clinical application and reducing the risk of contamination (Dohan Ehrenfest et al., 2009; Borie et al., 2015).

Despite the individual advantages of PRP and PRF, interest in combining both is growing to leverage their complementary properties. PRP's immediate bioactivity and PRF's extended-release profile may produce a synergistic effect that enhances bone regeneration more effectively than either agent alone. However, studies comparing the isolated and combined effects of PRP and PRF, particularly using radiological and biomechanical assessments, remain limited (Dohan Ehrenfest et al., 2009). This is especially true for critical-sized bone defects, where natural healing is often inadequate without intervention.

The present study addresses this gap by evaluating the comparative and combined effects of PRP and PRF on bone regeneration in a rabbit model of critical ulna defect. This research aims to provide foundational insights into the clinical utility of these biomaterials for enhancing long bone healing.

2. MATERIALS AND METHODS

2.1. Study Design and Animals

This experimental study used 28 adult New Zealand White rabbits, each weighing 2.5-3.5 kg and aged 5-6 months. The rabbits were acquired from the Animal Resource Facility, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto. All animals were apparently healthy and acclimatized for two weeks prior to the experiment. The rabbits were randomly assigned into four equal groups (n = 7) per group: Group A (PRP-treated), Group B (PRF-treated), Group C (PRP + PRF combination), and Group D (control; untreated).

2.2. Preparation of Platelet-rich Plasma (PRP) and Platelet-rich Fibrin (PRF)

For the PRP preparation, approximately 10 mL of whole blood was drawn from each rabbit's auricular vein into sterile tubes containing 3.8% sodium citrate (Chengdu Rich Science Industry Co., Ltd., Chengdu, China). A two-step centrifugation method was employed according to the standard procedure described by Dhurat & Sukesh (2014) and Reksodiputro et al. (2021): the first spin at 1600 rpm for 10 minutes separated the plasma, which was collected and centrifuged again at 3000 rpm for 10 minutes to concentrate the platelets. The resulting PRP fraction was extracted and activated with 10% calcium chloride (Hebei Huachen Pharmaceutical Group Co., Ltd., Cangzhou, Hebei, China) immediately before use. For the PRF, 10 mL of whole blood was collected into plain sterile glass tubes without anticoagulant and immediately centrifuged at 3000 rpm for 10 minutes following Choukroun's method. The PRF clot was then harvested from the middle layer, situated between the red blood cell layer and the acellular plasma.

2.3. Surgical Procedure and Postoperative Management

Under general anesthesia induced by using a combination of xylazine (Bela-Pharm, Vechta, Germany) (1 mg/kg IM) and ketamine (Vedco Inc., St. Joseph, MO, USA) (35 mg/kg IM), the right radius and ulna of each rabbit were clipped and aseptically prepared. The radius and ulna bones were surgically exposed as described by Hak et al. (2014). A 15-mm critical-sized defect was created at the mid-diaphyseal ulna using a dental burr under constant saline irrigation. In Group A, the defect was filled with 0.75 mL autologous PRP. In Group B, a 15-mm PRF clot was placed into the defect. In Group C, a mixture of 0.5 PRP and 10 mm PRF was implanted, while in Group D, the defect was left untreated. The wounds were closed in layers. Postoperative care included intramuscular administration of amoxicillin (10 mg/kg) for 5 days and diclofenac (0.2 mg/kg) for 3 days. Rabbits were housed individually and monitored daily for signs of infection or pain.

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2.4. Radiological Evaluation

Standard cranio-caudal and lateral radiographic views were taken at 0, 4, 8, and 12 weeks post-operative, using a digital radiography system (Shimadzu MobileDaRt Evolution MX8, Shimadzu Corp., Kyoto, Japan). Bone healing was assessed based on callus formation, defect bridging, and cortical continuity using a semi-quantitative scoring system as described by Oryan et al. (2015) and Liu et al. (2022a). The semi-quantitative radiographic scores for callus formation, defect bridging, and cortical continuity were expressed as mean ± SD for each treatment group at weeks 4, 8, and 12 post-surgery.

2.5. Biomechanical Testing

At 12 weeks post-operation, the animals were humanely euthanized using an overdose of pentobarbital sodium administered via the intraperitoneal (IP) route, in accordance with standard euthanasia protocols as described by the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals: 2020 Edition. Following euthanasia, the ulna bones were carefully harvested, and all surrounding soft tissues and muscle mass were meticulously removed. Biomechanical evaluation of the regenerated bone was performed using a Vickers microhardness test with a calibrated digital microhardness tester. The test was conducted following standard procedures described by ASTM E384-22 to determine the Vickers Hardness Number (VHN). Indentations were made using a diamond pyramid indenter with a load of 50 gf and a dwell time of 15 s to assess resistance to plastic deformation, reflecting local mineralization and tissue maturity in the healed bone.

2.6. Data Analysis

Semi-quantitative radiographic scores generated from the assessment of callus formation, defect bridging, and cortical continuity were expressed as Mean ± SD for each group at the respective time points. We compared the four experimental groups (PRP, PRF, PRP+PRF, and control) to assess differences in bone healing progression. We compared radiographic healing scores among groups at each time point to evaluate treatment-related differences in bone regeneration, as well as within groups over time to assess healing progression. Statistical analysis was performed using appropriate repeated-measures or generalized linear mixed-model approach to account for the effects of treatment and time. Where significant overall differences were detected, post-hoc multiple comparisons were conducted to identify specific group differences. This approach allowed objective comparison of the extent and rate of bone regeneration among PRP, PRF, PRP+PRF, and control groups over the experimental period.

Biomechanical test data obtained from three-point bending analysis were also expressed as mean ± SD, with corresponding minimum and maximum values calculated for each group. These parameters were compared across the four groups to determine treatment-related differences in bone strength and mineralization. Statistical analysis was carried out using one-way analysis of variance (ANOVA) to assess differences among groups. When significant differences were detected, Tukey's post hoc multiple comparisons test was applied to identify specific intergroup differences. Statistical significance was set at $P<0.05$. All analyses were performed using SPSS version 29.0 (IBM Corp., Armonk, NY, USA).

3. RESULTS

3.1. Radiographic Healing Assessment

Radiographic evaluation demonstrated progressive bone healing across all groups, although the rate and extent of regeneration varied with treatment. At week 0, all groups showed a sharply defined critical-sized ulna defect with no evidence of periosteal reaction or callus formation, confirming uniform defect creation (Fig. 1). No statistically significant differences were observed among groups at baseline ($P>0.05$).

By week 4, early healing differences became evident. Group A (PRP) exhibited faint periosteal reaction and early callus formation. Group B (PRF) showed more advanced healing with visible partial cortical bridging and denser callus formation. Group C (PRP+PRF) demonstrated early periosteal activity and callus formation comparable to PRF. In contrast, Group D (control) showed minimal to no periosteal response, with clearly demarcated defect margins (Fig. 1). Radiographic scores in the PRF and PRP+PRF groups were significantly higher than those of the PRP and control groups ($P<0.05$), while all treated groups scored significantly higher than the control ($P<0.001$); Table 1.

At week 8, healing progressed further in the treated groups (Table 1). The PRP group displayed partial cortical bridging with moderate callus development. Both PRF and PRP+PRF groups exhibited marked cortical bridging and increased callus density, indicating more advanced bone regeneration. The control group showed only faint callus formation without effective bridging (Fig. 1). Radiographic scores for PRF and PRP+PRF were significantly higher than those of PRP ($P<0.05$) and the control group ($P<0.001$), while PRP also differed significantly from the control ($P<0.05$) (Table 1).

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Table 1: Radiographic healing scores at 4, 8, and 12 weeks post-operation

Group	N=28	Week 4	Week 8	Week 12
Group A (PRP)	7	2.1 ± 0.4*	4.8 ± 0.6*	7.5 ± 0.8*
Group B (PRF)	7	2.8 ± 0.5*	5.5 ± 0.7*	8.8 ± 0.9*
Group C (Combination)	7	4.2 ± 0.6**	7.9 ± 0.8**	10.5 ± 1.1**
Group D (Control)	7	0.8 ± 0.2	1.5 ± 0.3	2.4 ± 0.5

Differences were considered statistically significant at P<0.05. Symbols (*, **, and ***) indicate statistically significant differences relative to the control group or among treatment groups, with higher scores reflecting improved radiographic bone healing.

By week 12, the PRP group showed near-complete cortical continuity, reflecting steady but moderate healing. Complete cortical continuity was evident in both PRF and PRP+PRF groups, whereas the control group demonstrated only partial bridging with persistent defect gaps. Radiographic scores were highest (Fig. 1) in the PRF and PRP+PRF groups and were significantly greater than those of the PRP and control groups (P<0.001). No statistically significant difference was detected between the PRF and PRP+PRF groups at this time point (P>0.05) (Table 1).

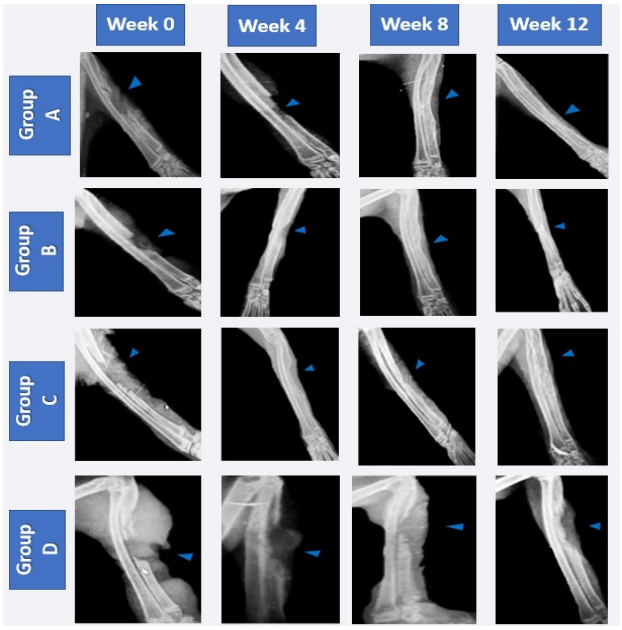


Fig. 1: Radiographic assessment of the healing of critical ulna defects in groups A (PRP), B (PRF), C (PRP+PRF), and D (Control) at weeks 0, 4, 8, and 12.

3.2. Biomechanical Strength

At 12 weeks post-surgery, Vickers hardness testing was performed to evaluate bone mineralization across the experimental groups. The control group (Group D) had the lowest mean hardness (118.3 ± 1.53), with a narrow range of 117–120 HV. This suggested poor and consistently low mineralization in untreated defects. In contrast, Group A (PRP alone) showed moderate improvement, with a mean hardness of 129.3 ± 5.47 and a broader range of 123–136 HV, reflecting more variable mineralization outcomes (Table 2).

Group B (PRF-treated alone) showed a significantly higher mean hardness of 145.5 ± 2.43, with a tighter range of 142–149 HV, indicating more consistent and elevated bone mineralization. Group C (PRP+PRF) recorded the highest hardness values, with a mean of 158.2 ± 5.95 and a range of 151–165 HV, clearly reflecting the most advanced mineralization among all groups (Table 2).

Welch's one-way ANOVA showed significant differences in hardness among groups (P=0.001). Post hoc analysis using the Tukey test revealed significant differences in mean hardness between Group C and Group D (P<0.001), and between Group C and Group A (P<0.001), indicating that the combination of PRP and PRF significantly enhanced bone hardness relative to both the untreated control and PRP alone. A borderline non-significant difference was observed between Group C and Group B (P=0.065), suggesting that PRP+PRF may offer a marginal improvement over PRF alone. Furthermore, Group B showed significantly higher hardness than the control (P<0.001) and moderately higher hardness than Group A (P=0.022). However, the difference between Group A and Group D was not significant (P=0.114), indicating that PRP alone did not markedly improve bone hardness compared to the untreated group.

Table 2: Biomechanical evaluation of bone mineralization using Vickers hardness (HV) at 12 weeks post-operation

Group	Treatment	Mean ± SD (HV)	Min HV	Max HV	Mineralization Trend
D	Control	118.3 ± 1.53	117	120	Predominantly Low
A	PRP	129.3 ± 5.47	123	136	Moderate(minimal Low)
B	PRF	145.5 ± 2.43	142	149	Good (consistent)
C	PRP+PRF	158.2 ± 5.95	151	165	High (optimal)

4. DISCUSSION

Radiographic assessment is a crucial method for monitoring non-invasive bone regeneration over time, providing valuable insight into the efficacy of different therapeutic approaches (Ahmad et al., 2025). This study found that while

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all treated groups showed progressive bone regeneration, the rate and extent of healing varied significantly among treatments. These findings are consistent with established principles that Platelet-derived growth factors positively influence osteogenesis (Nurden, 2018; Jia. et al., 2024).

Initially, defects in the PRP-treated group showed gradual but modest healing, with faint callus visible at week 4 that progressed to near-complete cortical continuity by week 12. This aligns with a moderate enhancement of bone regeneration over time. This outcome is consistent with previous studies, such as those by Abd-Alkhaleq & Hussein (2024) and Törün et al. (2025) in rabbit calvarial defects, which noted that PRP stimulates bone repair but often with less pronounced or sustained effects than other treatments. The transient nature of PRP's growth factor release likely explains these moderate outcomes, as highlighted by Dohan Ehrenfest et al. (2009) and Park et al. (2026).

In contrast, the PRF-treated group demonstrated more advanced and accelerated healing. Early periosteal activity and callus formation were evident by week 4, progressing to dense bone gap bridging at week 8 and complete cortical remodeling by week 12. This superior radiographic performance is supported by a wealth of literature, as reported by Hidajat et al. (2020) in a long bone defect model and Törün et al. (2025) in calvaria defects, both of which reported higher radiographic healing scores with PRF than with PRP or control groups. The sustained release of growth factors from PRF's fibrin matrix, which also acts as a biological scaffold, is believed to be the key factor enhancing early mineralized callus formation (Dohan Ehrenfest et al., 2009). Clinical evidence further corroborates this, with studies showing PRF improves radiographic bone density around implants (Al-Khawlani et al., 2014).

The combined PRP+PRF group also demonstrated strong regenerative effects, with early and sustained healing comparable to the PRF-only group. While the combination accelerated healing compared to PRP alone, it did not significantly outperform PRF at any stage. This aligns with findings from Törün et al. (2025), who found no additional benefit from combining platelet concentrate beyond that provided by PRF alone in their model. As Dohan Ehrenfest et al. (2009) have suggested, the significant biological overlap between PRP and PRF may limit a truly synergistic effect. However, the superior early radiographic healing of the combination group compared to PRP alone echoes findings by Pullishery et al. (2024), who observed enhanced radiographic bone density with combined therapies during early healing stages.

As expected, the untreated control group exhibited delayed and incomplete bone healing, confirming the critical-sized nature of the defect. Radiographs showed minimal healing progression throughout the observation period, consistent with findings from Nurden (2018) in similar animal models. This underscores the necessity of biological augmentation to achieve union in such defects.

The hardness test (HV) results further support these radiographic observations, revealing statistically significant differences in the quality of bone regeneration among treatment groups ($P=0.001$). This indicated that the type of platelet concentrates significantly influenced the mechanical strength of the newly formed bone. Post hoc Tukey's test showed that the combination group (PRP+PRF) had the highest mean hardness (158 ± 7), significantly greater than both the PRP-treated group ($P<0.001$) and the control group ($P<0.001$). The PRF-treated group also exhibited significantly higher hardness than PRP ($P=0.022$) and the control group ($P<0.001$). In contrast, no significant difference was observed between the PRP and control groups ($P=0.114$), suggesting that PRP alone did not significantly enhance mechanical strength within the study period.

These findings consistently highlight the superior mechanical properties achieved with combined PRP+PRF, supporting a synergistic interaction between the two platelet concentrates. This aligns with studies reporting enhanced biomechanical strength when PRP was combined with scaffolds (Oryan et al., 2012) or other bioactive materials, suggesting that combining growth factors with scaffold properties is key to improved outcomes (Patel et al., 2023). The lack of a significant improvement with PRP over the control group reinforces the observation that PRP's accelerated early healing effects may not be sustained long-term unless paired with an appropriate scaffold (Ghaffarpasand et al., 2016; Yaşar et al., 2025).

PRF's distinct advantage over PRP stems from its slow, steady release of growth factors from its fibrin matrix, as well as its role as a biological scaffold. This promotes osteoprogenitor cell recruitment and differentiation, leading to more organized and mineralized bone formation (Choukroun et al., 2006; Elmeshreghi et al., 2025).

Overall, the significant improvements in bone hardness in the PRF and PRP+PRF groups, along with the lack of enhancement in the PRP group compared to control, underscore PRF's critical role in improving biomechanical bone regeneration quality. These findings strongly align with the conclusions of Shafiei-Sarvestani et al. (2015); Bacevich et al. (2024); and Elsayed et al. (2025), who emphasized that combined platelet derivative use can yield superior histological and mechanical bone repair outcomes.

5. CONCLUSION

In conclusion, both the radiographic and biomechanical assessments suggest that PRF, particularly in combination with PRP, offers a more effective strategy for enhancing bone regeneration in orthopedic applications.

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The early advantage demonstrated by PRF, driven by its superior and sustained release of bioactive factors, provides compelling evidence of its potency during the critical early stages of healing.

Declarations

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Ethics Statement: Procedures in this study were performed in accordance with institutional animal care and use committee guidelines and with ethical approval from the Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria, with the approval number UDUS/IACUC/2025/R08.

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