# Bone Healing Enhancing with the Xenogenic (Horse) Lyophilized Leukocyte-and Platelet-Rich Fibrin (L-Prf) in Rat Animal Model

**Abstract**

**Introduction:** Bone defects resulting from trauma, pathological conditions, or surgical interventions often require advanced regenerative strategies, particularly when critical-sized defects exceed the body's intrinsic healing capacity. Among various biomaterials, Leukocyte- and Platelet-Rich Fibrin (L-PRF) has gained significant attention for its autologous origin, bioactive properties, and potential application in both autologous and xenogenic forms to enhance bone regeneration.

**Materials & Methods:** Adult male Wistar rats were randomly divided to four experimental groups to evaluate the effects of equine-derived lyophilized L-PRF, with or without autologous bone grafts, on critical-sized radial bone defects. L-PRF was prepared from equine blood, lyophilized, and applied to the defect sites, with bone healing assessed via radiography and histopathology, and statistical differences among groups analyzed using non-parametric methods.

**Results & Discussion:** Radiographic and histopathological analyses demonstrated that the autograft + L-PRF group exhibited the most pronounced bone healing, with significantly higher Lane and Sandhu scores and evidence of progressive cartilage-to-bone formation compared to the L-PRF alone and empty defect groups. The autograft group also showed superior outcomes relative to L-PRF alone, while untreated defects displayed primarily fibrous tissue with no bone formation, highlighting the synergistic effect of combining L-PRF with autologous bone grafts.

**Conclusion**: In this study, xenogenic equine-derived L-PRF enhanced the repair of critical-sized radial bone defects in rats, with its combination with autografts yielding superior osteogenic outcomes compared to L-PRF alone.

**Keywords:** Leukocyte-Platelet-Rich Fibrin (L-PRF), Xenogenic L-PRF, Bone healing, Critical defect, Rat, Radiology, Histopathology.

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## Introduction

Bone tissue can sustain damage due to numerous factors, including trauma, pathological conditions, and surgical interventions[1, 2]. The biological response to such injuries encompasses a highly regulated and multifaceted cascade involving the recruitment of cellular and molecular mediators essential for tissue regeneration and repair[3-5]. Advancements in bone regeneration research have been guided by the intrinsic mechanisms of physiological repair, employing autologous bioactive factors to enhance and expedite osteogenesis[6]. Recent studies have validated the effectiveness of biomaterials in facilitating the reconstruction of critical -sized osseous defects[7]. A critical-sized bone defect refers to a structural osseous defect that exceeds the intrinsic regenerative capacity of the body, rendering spontaneous healing impossible and requiring surgical intervention to restore bone continuity and function[8] Among the most promising scaffolding biomaterials, Leukocyte and Platelet-Rich Fibrin (L-PRF) has attracted considerable attention because of its autologous origin, bioactive properties, and extensive clinical applications, particularly in regenerative dentistry and oral surgeries[6, 9-17].

Fibrin represents the activated form of the fibrinogen molecule, playing a pivotal role in platelet aggregation and blood coagulation. studies have demonstrated that, through the centrifugation of blood, a biologically active product known as Leukocyte- and Platelet-Rich Fibrin (L-PRF) can be obtained, which significantly contributes to healing of wound and tissue regeneration . Leukocyte-and Platelet-Rich Fibrin (L-PRF) is a new platelet concentrates generation, distinguished by its preparation method that requires no anticoagulants or exogenous additives, thereby preserving its natural fibrin architecture and bioactive components[18]. A study conducted in 2012 demonstrated that Leukocyte-and Platelet-Rich Fibrin (L-PRF) is capable of achieving a substantial concentration of growth factors in its fibrin matrix, thereby enhancing its potential for application in orthopedic injuries and regenerative healing[19]. Moreover, evidence from a 2022 study indicated that Leukocyte- and Platelet-Rich Fibrin (L-PRF) plays a substantial role in scalp defect regeneration in humans, showing enhanced efficacy and superior clinical outcomes relative to other conventional regenerative approaches[20]. L-PRF has been shown to enhance regenerative outcomes when combined with adjunctive therapies. For instance, Sadeghian et al. reported a synergistic effect of L-PRF in conjunction with photobiomodulation therapy (PBMT), further accelerating the bone healing process[21]. Most experimental and clinical investigations have primarily focused on the application of autologous L-PRF; however, recent findings indicate that xenogeneic L-PRF may also increase the regenerative potential of critical-sized osseous defects[22]. In the present study, lyophilized L-PRF powder derived from equine blood was employed to investigate its potential effect on bone regeneration in critical-sized radial defects in a rat model. To date, the most recent research utilizing xenogenic L-PRF was reported by Khanbazi et al., in which bovine-derived L-PRF was used[22].

## Materials & Methods

### Animal Model

Adult male Wistar albino rats were used as the model in this study, as rats have been widely utilized due to their close physiological and biological similarities to humans and their suitability for monitoring bone healing processes[2]. A total of twenty (n=20) healthy adult rats, weighing 180–200 g, were selected.

### Animal Grouping and Housing

The rats were divided into four experimental groups randomly (5 in each group). After a one-day acclimatization period and confirmation of their health status, the animals were tagged and transferred to the Surgery Department, Faculty of Veterinary Medicine, Shiraz University. The study was performed in four groups: Group I (Negative Control): Bone defect without any treatment. Group II (Positive Control): Bone defect with autologous bone grafting harvested from the defect site. Group III: Bone defect treated with equine-derived Leukocyte-and Platelet-Rich Fibrin (L-PRF) powder combined with an autologous bone graft. Group IV: Bone defect treated with equine-derived Leukocyte-and Platelet-Rich Fibrin (L-PRF) powder alone.

### Preparation of Xenogeneic Leukocyte-Platelet-Rich Fibrin (L-PRF)

Approximately 20 mL of whole blood was achieved from a healthy domestic horse housed at the Shiraz University Veterinary School Stable. The sample was transferred into vacutainer tubes without anticoagulants and transported to the Veterinary Laboratory within less than one minute after collection. Following centrifugation (3000 rpm for 10 minutes), the samples separated into three distinct layers: The upper layer consisting of acellular plasma, The middle layer containing Leukocyte-and Platelet-Rich Fibrin (L-PRF), The lower containing erythrocytes. Using sterile forceps, the L-PRF clots were carefully harvested from the middle layer under aseptic conditions[18, 23].

### Preparation of Lyophilized L-PRF

Freshly prepared L-PRF clots were placed into sterile test tubes, ensuring that the clots filled approximately one-tenth of each tube's volume. The tubes were sealed with Parafilm—perforated to allow minimal air exchange—preventing spillage during the lyophilization process. The samples were stored in a cryogenic freezer at −80 °C overnight and subsequently lyophilized in Zirbus VaCo 5 Laboratory Freeze Dryer machine following the protocol described by Wang et al. [24] : Cold trap temperature: −45 °C ± 5 °C, Initial vacuum pressure: 0.1 mbar, Primary drying time: 17 h, Final vacuum pressure:

0.001 mbar, Secondary drying time: 8 h Upon completion of the lyophilization process, the resulting L-PRF powder was achieved and in sealed sterile tubes at room temperature for 24 hours stored for further use.

### Surgical Procedure

General anesthesia with injection of Ketamine (10%, Alfasan, Netherlands) — 10 mL, Acepromazine (1%, Alfasan, Netherlands) — 1 mL, Diazepam (10 mg) — 2 mL intramuscularly was achieved . A total volume of 0.3 mL of this mixture was administered intramuscularly to each rat. A longitudinal incision on craniolateral side of left forearm was done, and the mid-diaphysis of the radius was exposed. A 3-mm defect in the radial bone with using a 3-mm drill bit was created, while the average radial shaft diameter was approximately 1.2 mm. The animals were allocated into four experimental groups as described previously. The incision site was closed using a single cruciate suture pattern under sterile conditions. To prevent potential postoperative infection, 1 mL of enrofloxacin (10%) diluted in distilled water (Enrofan 5%, Erfan, Tehran, Iran) was administered to each animal.

### Radiological Assessment

After anesthesia was made in rats, radiographic images of the left forelimbs of the rats were obtained postoperatively on days 35 and 64 in the lateral projection (Varian Medical Systems). The obtained radiographs were evaluated using the modified Lane and Sandhu scoring system[25].

### Histopathological Assessment

On day 64, all rats were humanely euthanized, and bone samples were sent to the specialized pathology laboratory for histopathological examination. After dehydration, clearing, and embedding, 5 µm thickness of tissues were stained with hematoxylin and eosin (H&E) to obtain histological slides.
The veterinary pathologist assessed the slides with the Emery's scoring system[26] for bone healing as summarized in Table 1.

### Statistical Analysis

The collected data were systematically organized using Microsoft Excel, tables were prepared in Microsoft Word, and all datasets were subsequently analyzed statistically using the Kruskal–Wallis non-parametric one-way ANOVA to assess differences among the experimental groups. When a statistically significant difference was detected (≤ 0.05), pairwise comparisons were performed using the Mann–Whitney U test. A p-value ≤ 0.05 was considered statistically significant. All statistical analyses were conducted using SPSS statistical software (Version 26, IBM Corp., Armonk, NY, USA).

## Results

### Clinical evaluation

There was no death and no ulnar fracture in the study period .

### Radiographic findings:

The results of the radiological assessment of proximal union, distal union, bone formation, and remodeling are presented in Table 2.
As shown in Table 2, the empty defect group demonstrated significantly inferior performance compared to both the autograft and the autograft + L-PRF group at 35 and 64 days ($p \leq 0.05$). On day 35, the autograft group had significantly better outcome compared to the L-PRF ($p \leq 0.05$). Moreover, at both day 35 and day 64, the autograft + L-PRF group showed markedly superior performance compared to the L-PRF group, achieving a median Lane and Sandhu score of 8. These results indicate that autograft + L-PRF group exhibited a superior bone healing response. There is no statistically significant differences between the other groups ($p > 0.05$).

### Histopathological Findings

Histopathological evaluation conducted at 64 days postoperatively revealed the following findings. In the group with empty defect, irregular loose connective tissue was observed at the bone defect. The defect margins were primarily composed of fibroblasts, fibrocytes, and collagen, with no sign of formation of bone or cartilage in the untreated lesions(Figure 1). In the autograft group, well-developed cartilage with clearly visible chondrocytes was observed along with early signs of compact bone formation, including the presence of Haversian canals (Figure 2). In the autograft + L-PRF group, immature bone tissue was formed, with a pronounced progression from cartilage to bone (Figure 3). In the L-PRF group, connective tissue and cartilage were

present at the defect site, with ongoing development of trabecular bone(Figure 4). The results of the histopathological evaluation across the experimental groups are presented in Table 3. Data were initially analyzed and statistically significant differences were observed ($p \leq 0.05$), Empty defect group had a statistically significant difference compared with the autograft group ($p = 0.01$) or the autograft + L-PRF group ($p = 0.08$), demonstrating markedly inferior performance in both comparisons.

**Table 1: Emery's Histological Bone Healing Scoring System**

| Score | Dominant Tissue Type | Histological Description |
|---|---|---|
| 0 | Empty defect | No tissue formation; the defect remains unfilled. |
| 1 | Predominantly fibrous connective tissue | Defect filled mainly with fibrous tissue; minimal cellular organization. |
| 2 | Fibrous tissue > cartilage | Fibrous connective tissue dominates; small islands of immature cartilage observed. |
| 3 | Cartilage > fibrous tissue | Cartilaginous matrix becomes more pronounced, replacing most of the fibrous tissue. |
| 4 | Pure cartilage | Defect filled entirely with hyaline-like cartilage; no evidence of ossification. |
| 5 | Cartilage > bone | Early endochondral ossification; cartilage partially replaced by immature woven bone. |
| 6 | Bone > cartilage | Woven bone dominates with small residual cartilaginous areas; advancing ossification. |
| 7 | Pure bone | Defect completely bridged by mature bone; full osseous healing achieved. |

**Table 2: Radiographic Evaluation of results**

| Day | Group-I (Empty Defect) *Median (Range)* | Group-II (Autograft) *Median (Range)* | Group III (Autograft + L-PRF) *Median (Range)* | Group-IV (L-PRF) *Median (Range)* | p-value |
|---|---|---|---|---|---|
| Day 35 | 3 *(1–3)** | 7 *(5–8)** | 8 *(6–8)** | 4 *(3–4)* | 0.004 |
| Day 64 | 4 *(4–5)** | 7 *(4–8)* | 8 *(8–8)** | 5 *(4–7)* | 0.010 |

Values are presented as median (minimum–maximum). * show statistically significant differences between groups ($p \leq 0.05$).

**Table 3: Histopathological evaluation of results**

| Group | Empty defect | Autograft | L-PRF | Autograft + L-PRF | *p*-value |
|---|---|---|---|---|---|
| Histopathological bone healing score (Day 64) (Median [range]) | 1 (1–3)[b] | 6 (5–6) | 3 (3–4) | 5 (4–5) | 0.003 |

Values are presented as median (minimum–maximum). Superscript letter ([b]) shows statistically significant difference between groups ($p \leq 0.05$).

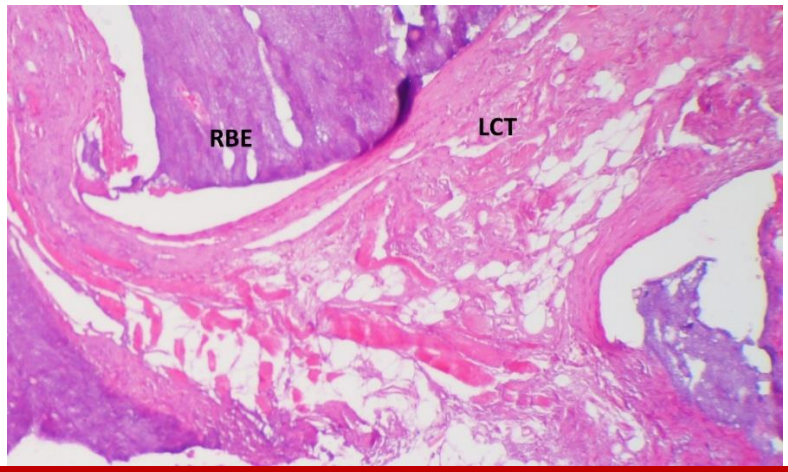


**Figure 1: Histopathological image of the untreated group defect site. RBE: radial bone edge, LCT: loose connective tissue. (H&E. ×40)**

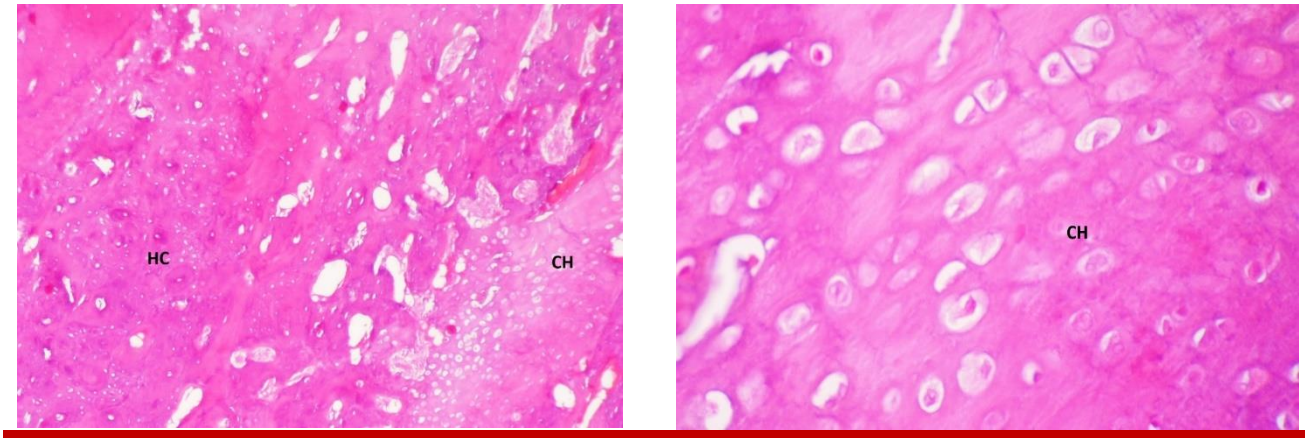


**Figure 2: Histopathological images of the autograft group defect site. HC: Haversian canal, CH: chondrocyte. (H&E. ×40, ×400)**

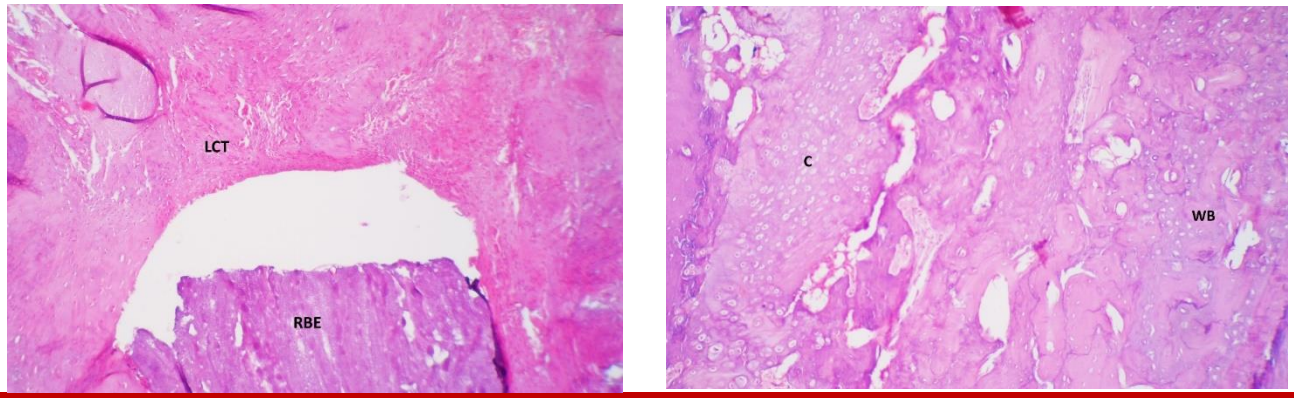


**Figure 3: Histopathological images of the autograft + L-PRF group defect site. LCT: loose connective tissue, RBE: radial bone edge, C: cartilage, WB: woven bone. (H&E. ×100)**

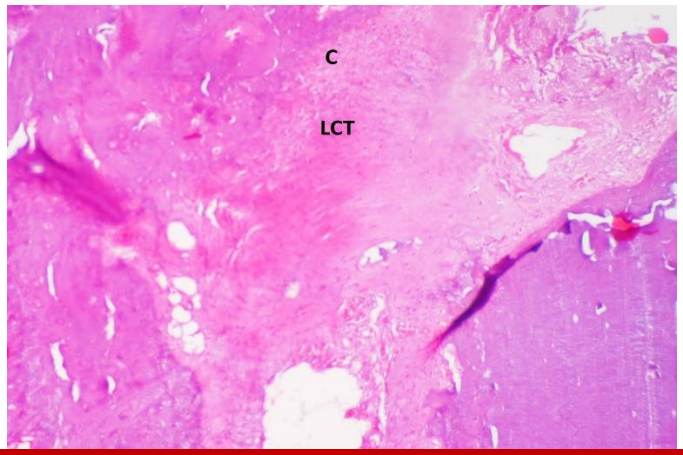


**Figure 4: Histopathological image of the L-PRF group defect site. LCT: loose connective tissue, C: cartilage. (H&E. ×40, ×400)**

## Discussion

Leukocyte and Platelet-Rich Fibrin (L-PRF) offers distinct advantages over earlier platelet concentrating systems, as it is prepared without anticoagulants or additives and forms a dense fibrin matrix that gradually releases cytokines while supporting cell migration. Its simple, low-cost, and reproducible protocol enables the production of multiple clots, while the inclusion of leukocytes enhances angiogenesis and immune modulation, making L-PRF a clinically versatile biomaterial[27].

Leukocyte-and Platelet-Rich Fibrin (L-PRF) forms a dense, well-polymerized fibrin matrix containing a viable population of leukocytes and platelets, which ensures structural integrity and for at least seven days releasing of growth factors like TGF-β1, PDGF-AB, VEGF, and BMP-2 significantly increased [28, 29].

The fibrin network formed during centrifugation exhibits a three-dimensional, permeable architecture that supports cellular colonization and accelerates wound and bone healing when combined with grafts. As an autologous biomaterial, L-PRF eliminates risks of infection or disease transmission[30] Also, Its dense, elastic fibrin matrix supports cell adhesion and proliferation while modulating inflammation and macrophage polarization toward a regenerative M2 phenotype. With minimal immunogenicity and excellent handling properties, L-PRF facilitates soft tissue and hard tissue repair, and that makes it as a valuable tool in regenerative medicine.[31]

The present study demonstrates the effect of L-PRF in stimulating the repair of critical-sized radius defects in rats, as confirmed by both radiological and histopathological analyses. In this study, equine blood was utilized to produce a novel xenogenic L-PRF, and the results indicate its significant potential in enhancing bone regeneration. As demonstrated in numerous previous studies, L-PRF exerts a positive impact on healing of bone [32-35], and therefore unsurprising that a similar regenerative response was observed in the present investigation. Consistent with previous reports, fibrin-based platelet concentrates and L-PRF membranes provide a supportive, biologically active three-dimensional matrix that promotes cell adhesion, proliferation, angiogenesis, and sustained release of bioactive molecules while exerting antibacterial and analgesic effects. These properties align with our findings, highlighting the potential for regeneration of L-PRF in enhancing the repair of challenging tissue defects, including bone lesions[36, 37]. While previous studies utilized other forms of L-PRF, we employed lyophilized L-PRF, which, as Qi Li et al. demonstrated, provides improved shelf-life, enhanced osteogenic potential, and better tissue integration in comparison to fresh PRF[38].

Previous study showed that worked on periodontal regeneration, indicate that incorporating PRF with open flap debridement (OFD) enhances vertical clinical attachment levels (VCAL) compared to horizontal clinical attachment levels (HCAL) and radiographic bone fill (RFB) outcomes in managing class II furcation defects compared to OFD alone. Moreover, the combination of bone grafts (BG) and PRF, results in statistically significant additional improvements in VCAL/HCAL[39]. As in oral surgery, L-PRF also been used in cranial surgery and demonstrated comparable effectiveness to commercial fibrin sealants for dural reinforcement without any safety concerns, and its integration into clinical practice may offer significant cost advantages due to its affordability and availability[40]. Despite variations among studies, evidence shows that PRF enhances key cellular processes involved in anti-inflammation process[41] and, whether used alone or in combination with metronidazole, effectively contributes to the successful management of moderate periodontitis[42].

Juan Blanco et al. suggests the effectiveness of L-PRF on bone tissue regeneration remains debatable and requires further investigation, nonetheless, its positive impact on soft tissue healing has been well established[43]. also the study of Fernanda Faot et al., showed that L-PRF can not improve the non-critical bone defects healing in the rabbit tibia[44].

A study have indicated that the osteogenic effectiveness of L-PRF on bone repair can be significantly enhanced when applied in combination with an additional reparative factor, suggesting that its therapeutic potential may be greater in a synergistic context than when used alone[45]. As José Augusto Gabarra Júnior et al. suggested that the combination of nanohydroxyapatite with L-PRF promote better bone formation compared to other tested composite materials[46]. Our findings are consistent with this concept, as the autograft + L-PRF group exhibited greater bone regeneration compared to the L-PRF-only group, indicating that combining L-PRF with an additional reparative factor can enhance osteogenic outcomes. This is in line with numerous studies demonstrating that autografts alone are also capable of accelerating bone formation at defect sites[47-49].

Emerging evidence suggests that alternative platelet-rich fibrin formulations, including injectable platelet-rich fibrin (i-PRF) and advanced platelet-rich fibrin (A-PRF) may outperform conventional L-PRF in the sustainable growth factors release and stimulation of osteogenesis.[50, 51]

Consequently, future investigations should prioritize these advanced PRF variants and rigorously evaluate their synergistic potential when combined with established regenerative modalities, such as

autologous bone grafts, to optimize bone healing outcomes.

## Conclusion

lyophilized L-PRF is a versatile autologous biomaterial that enhances tissue regeneration through its dense fibrin matrix, sustained growth factor release, and modulation of inflammation, angiogenesis, and cell proliferation. This study showed that xenogenic lyophilized L-PRF derived from equine blood had a positive impact on the repair of critical-sized radius defects in rats. Moreover, combination of L-PRF with autografts leads to superior healing in bone in comparison to the use of L-PRF alone, indicating a synergistic effect that enhances osteogenic outcomes.

## References

1 Sathyendra V, Darowish M. Basic science of bone healing. Hand clinics. 2013;29(4):473–81.
2 Oryan A, Monazzah S, Bigham-Sadegh A. Bone injury and fracture healing biology. Biomedical and environmental sciences. 2015;28(1):57–71.
3 Florencio-Silva R, Sasso GRdS, Sasso-Cerri E, Simões MJ, Cerri PS. Biology of bone tissue: structure, function, and factors that influence bone cells. BioMed research international. 2015;2015(1):421746.
4 Loi F, Córdova LA, Pajarinen J, Lin T-h, Yao Z, Goodman SB. Inflammation, fracture and bone repair. Bone. 2016;86:119–30.
5 Schini M, Vilaca T, Gossiel F, Salam S, Eastell R. Bone turnover markers: basic biology to clinical applications. Endocrine Reviews. 2023;44(3):417–73.
6 Coucke B, Dilissen E, Cremer J, Schrijvers R, Theys T, Van Gerven L. Leukocyte-and Platelet-Rich Fibrin for enhanced tissue repair: an in vitro study characterizing cellular composition, growth factor kinetics and transcriptomic insights. Molecular Biology Reports. 2024;51(1):954.
7 Călin C, Dima O, Ancuta DL, Coman C, Pameijer CH, Paulsen F, et al. Guided bone regeneration using beta-tricalcium phosphate and leucocyte platelet-rich fibrin versus a novel biodegradable urethane composite in critical-size osseous defects in rabbit tibia: histologic results of a pilot study. Annals of Anatomy-Anatomischer Anzeiger. 2025:152672.
8 Nauth A, Schemitsch E, Norris B, Nollin Z, Watson JT. Critical-size bone defects: is there a consensus for diagnosis and treatment? Journal of orthopaedic trauma. 2018;32:S7–S11.
9 Mudalal M, Wang Z, Mustafa S, Liu Y, Wang Y, Yu J, et al. Effect of Leukocyte-Platelet Rich Fibrin (L-PRF) on tissue regeneration and proliferation of human gingival fibroblast cells cultured using a modified method. Tissue Engineering and Regenerative Medicine. 2021;18(5):895–904.
10 Xu J, Ren H, Zhao S, Li Q, Li C, Bao G, et al. Comparative effectiveness of hyaluronic acid, platelet-rich plasma, and platelet-rich fibrin in treating temporomandibular disorders: a systematic review and network meta-analysis. Head & Face Medicine. 2023;19(1):39.
11 Miron RJ, Fujioka-Kobayashi M, Sculean A, Zhang Y. Optimization of platelet-rich fibrin. Periodontology 2000. 2024;94(1):79–91.
12 Egierska D, Perszke M, Mazur M, Duś-Ilnicka I. Platelet-rich plasma and platelet-rich fibrin in oral surgery: A narrative review. Dental and Medical Problems. 2023;60(1):177–86.
13 Moraes RP, Costa FWG, SILVA PGdB, Carvalho FSR, Paz JERM, Matos GC, et al. Impact of L-PRF on pain and healing outcomes in lower third molar surgery: a randomized split-mouth trial. Brazilian Oral Research. 2024;38:e089.
14 Demirok SO, Eroglu CN, Koc A. Comprehensive analysis of bone tissue in extraction sockets of third molars after leukocyte and platelet rich fibrin and photobiomodulation applications. Clinical Oral Investigations. 2024;28(9):483.
15 Chmielewski M, Pilloni A, Adamska P. Application of Advanced Platelet-Rich Fibrin in Oral and Maxillo-Facial Surgery: A Systematic Review. Journal of Functional Biomaterials. 2024;15(12):377.
16 Almutairi M, Alshammari SZ, Almutairi D, Alansari TA, Aldhafiri F, Almutairi AN, et al. Leukocyte-Platelet-Rich Fibrin Combined With Demineralized Bovine Bone Mineral for Maxillary Sinus Augmentation: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Cureus. 2025;17(6).
17 Medeiros-Monzón A, Blanco-Carrión A, Gándara-Vila P, Camolesi G, Pérez-Jardón A, Lorenzo-Pouso AI, et al. Efficacy of leukocyte-and platelet-rich fibrin in the treatment and prevention of medication-related osteonecrosis of the jaw: a prospective study. Medicina oral, patologia oral y cirugia bucal.27249.
18 Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I: technological concepts and evolution. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2006;101(3):e37–e44.
19 A Zumstein M, Berger S, Schober M, Boileau P, W Nyffeler R, Horn M, et al. Leukocyte-and platelet-rich fibrin (L-PRF) for long-term delivery of growth factor in rotator cuff repair: review, preliminary results and future directions. Current pharmaceutical biotechnology. 2012;13(7):1196–206.
20 Van Cleemput T, Hendrikx S, Politis C, Spaey Y. Leukocyte- and platelet-rich fibrin: a new method for scalp defect reconstruction. Dermatologic Surgery. 2022;48(2):261–2.
21 Sadeghian A, Rohani B, Hasannia S, Mashhadiabbas F, Fekrazad R. Synergistic Effect of Photobiomodulation and Leukocyte-Platelet Rich Fibrin on Bone Regeneration in Rat Critical Defects. Photodiagnosis and Photodynamic Therapy. 2025:104687.
22 Khanbazi M, Bigham-Sadegh A, Oryan A, Meimandi-Parizi A, Jannesar A. The effects of allogeneic and xenogeneic lyophilized leukocyte-and platelet-rich fibrin on bone healing in rat. Injury. 2024;55(3):111396.
23 Crisci A, Lombardi D, Serra E, Lombardi G, Cardillo F, Crisci M. Standardized protocol proposed for clinical use of L-PRF and the use of L-PRF Wound Box®. Journal of Unexplored Medical Data¦ Volume. 2017;2:78.
24 Wang Z, Han L, Sun T, Wang W, Li X, Wu B. Preparation and effect of lyophilized platelet-rich fibrin on the osteogenic potential of bone marrow mesenchymal stem cells in vitro and in vivo. Heliyon. 2019;5(10).

25 Lane JM, Sandhu HS. Current approaches to experimental bone grafting. Orthopedic Clinics of North America. 1987;18(2):213–25.
26 Emery SE, Brazinski MS, Koka A, Bensusan JS, Stevenson S. The biological and biomechanical effects of irradiation on anterior spinal bone grafts in a canine model. JBJS. 1994;76(4):540–8.
27 Ehrenfest DMD, Rasmusson L, Albrektsson T. Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte-and platelet-rich fibrin (L-PRF). Trends in biotechnology. 2009;27(3):158–67.
28 Tablin F, Walker NJ, Hogle SE, Pratt SM, Norris JW. Assessment of platelet growth factors in supernatants from rehydrated freeze-dried equine platelets and their effects on fibroblasts in vitro. American journal of veterinary research. 2008;69(11):1512–9.
29 Dohan Ehrenfest DM, Pinto NR, Pereda A, Jiménez P, Corso MD, Kang B-S, et al. The impact of the centrifuge characteristics and centrifugation protocols on the cells, growth factors, and fibrin architecture of a leukocyte-and platelet-rich fibrin (L-PRF) clot and membrane. Platelets. 2018;29(2):171–84.
30 INCHINGOLO F, Tatullo M, Marrelli M, Inchingolo A, SCACCO S, Inchingolo A, et al. Trial with Platelet-Rich Fibrin and Bio-Oss used as grafting materials in the treatment of the severe maxillar bone atrophy: clinical and radiological evaluations. European Review for Medical & Pharmacological Sciences. 2010;14(12).
31 Quirynen M, Blanco J, Wang HL, Donos N, Temmerman A, Castro A, et al. Instructions for the use of L-PRF in different clinical indications. Periodontology 2000. 2025;97(1):420–32.
32 Ehrenfest DMD, Diss A, Odin G, Doglioli P, Hippolyte M-P, Charrier J-B. In vitro effects of Choukroun's PRF (platelet-rich fibrin) on human gingival fibroblasts, dermal prekeratinocytes, preadipocytes, and maxillofacial osteoblasts in primary cultures. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2009;108(3):341–52.
33 Ehrenfest DMD, Doglioli P, de Peppo GM, Del Corso M, Charrier J-B. Choukroun's platelet-rich fibrin (PRF) stimulates in vitro proliferation and differentiation of human oral bone mesenchymal stem cell in a dose-dependent way. Archives of oral biology. 2010;55(3):185–94.
34 Chang IC, Tsai CH, Chang YC. Platelet-rich fibrin modulates the expression of extracellular signal-regulated protein kinase and osteoprotegerin in human osteoblasts. Journal of biomedical materials research Part A. 2010;95(1):327–32.
35 Clipet F, Tricot S, Alno N, Massot M, Solhi H, Cathelineau G, et al. In vitro effects of Choukroun's platelet-rich fibrin conditioned medium on 3 different cell lines implicated in dental implantology. Implant dentistry. 2012;21(1):51–6.
36 Everts PA, Lana JF, Alexander RW, Dallo I, Kon E, Ambach MA, et al. Profound properties of protein-rich, platelet-rich plasma matrices as novel, multi-purpose biological platforms in tissue repair, regeneration, and wound healing. International Journal of Molecular Sciences. 2024;25(14):7914.
37 Pinto N, Yu J, Koirala S, Mourão CF, Andrade C, Rescigno E, et al. L-PRF in extra-oral wound care. Periodontology 2000. 2025;97(1):342–62.
38 Li Q, Reed DA, Min L, Gopinathan G, Li S, Dangaria SJ, et al. Lyophilized platelet-rich fibrin (PRF) promotes craniofacial bone regeneration through Runx2. International journal of molecular sciences. 2014;15(5):8509–25.
39 Miron RJ, Moraschini V, Estrin NE, Shibli JA, Cosgarea R, Jepsen K, et al. Periodontal Regeneration using Platelet-Rich Fibrin. Furcation defects: a systematic review with meta-analysis. Periodontology 2000. 2025;97(1):191–214.
40 Coucke B, De Vleeschouwer S, van Loon J, Van Calenbergh F, Van Hoylandt A, Van Gerven L, et al. Leukocyte-and platelet-rich fibrin in cranial surgery: a single-blinded, prospective, randomized controlled noninferiority trial. Journal of Neurosurgery. 2024;141(2):500–8.
41 Strauss F-J, Nasirzade J, Kargarpoor Z, Stähli A, Gruber R. Effect of platelet-rich fibrin on cell proliferation, migration, differentiation, inflammation, and osteoclastogenesis: a systematic review of in vitro studies. Clinical oral investigations. 2020;24(2):569–84.
42 Omar YK, Rashidy MAE, Ahmed GB, Aboulela AG. Evaluation of leukocyte-platelet rich fibrin as an antibiotic slow-release biological device in the treatment of moderate periodontitis: a randomized controlled clinical trial. BMC Oral Health. 2024;24(1):1530.
43 Blanco J, García A, Hermida-Nogueira L, Castro AB. How to explain the beneficial effects of leukocyte-and platelet-rich fibrin. Periodontology 2000. 2025;97(1):74–94.
44 Faot F, Deprez S, Vandamme K, Camargos GV, Pinto N, Wouters J, et al. The effect of L-PRF membranes on bone healing in rabbit tibiae bone defects: micro-CT and biomarker results. Scientific reports. 2017;7(1):46452.
45 Idulhaq M, Mudigdo A, Utomo P, Wasita B, Warman FI. Platelet-rich fibrin as a tissue engineering material in accelerate bone healing in rat bone defects: A systematic review and meta-analysis. Annals of Medicine and Surgery. 2022;84:104869.
46 Júnior JAG, Nóbrega F, Oliveira PG, Bergamo ET, Cadore U, Gomes MZdV, et al. Evaluation of Implant Surface Modification with Nanohydroxyapatite Associated with the Use of L-PRF: In Vivo Study in Rats. Journal of Functional Biomaterials. 2023;14(7):370.
47 Jensen SS, Broggini N, Hjørting-Hansen E, Schenk R, Buser D. Bone healing and graft resorption of autograft, anorganic bovine bone and β-tricalcium phosphate. A histologic and histomorphometric study in the mandibles of minipigs. Clinical oral implants research. 2006;17(3):237–43.
48 Marino JT, Ziran BH. Use of solid and cancellous autologous bone graft for fractures and nonunions. Orthopedic Clinics. 2010;41(1):15–26.
49 Azi ML, Aprato A, Santi I, Kfuri Jr M, Masse A, Joeris A. Autologous bone graft in the treatment of post-traumatic bone defects: a systematic review and meta-analysis. BMC Musculoskeletal Disorders. 2016;17(1):465.
50 Farshidfar N, Amiri MA, Estrin NE, Ahmad P, Sculean A, Zhang Y, et al. Platelet-rich plasma (PRP) versus injectable platelet-rich fibrin (i-PRF): A systematic review across all fields of medicine. Periodontology 2000. 2025.
51 Ravi S, Santhanakrishnan M. Mechanical, chemical, structural analysis and comparative release of PDGF-AA from L-PRF, A-PRF and T-PRF-an in vitro study. Biomaterials Research. 2020;24(1):16.