URF PUBLISHERS
connect with research world

Journal of Cell Science and Regenerative Medicine

https://urfpublishers.com/journal/cell-science-regenerative-medicine

Volume 1, Issue 2 (June) 2025

Research Article

A Breakthrough for Regenerative Medicine! Standardized Low-Cost Platelet-Rich Plasma Preparation in a Closed System (PRP-LCCS)

Palmerindo Antônio Tavares de Mendonça Néto[1]*, Mayara Magda Dantas Tavares de Mendonça[1], Dirceu Moraes Junior[2], Carlos Stéfano Hoffmann Brito[3], Leonardo Lemos Rebouças[4], Radanezi Potengy Junior[5], Gustavo Costa Rios[6], Joel Dourado Franco[3] and Daniel Ramos Gonçalves Lopes[7]

[1]Instituto Regenera Dor, Juazeiro do Norte, Ceará, Brazil

[2]Lumius Clinic, Joinville, Santa Catarina, Brazil

[3]Carlos Stéfano Institute, Belo Horizonte, Minas Gerais, Brazil

[4]Interpain, Fortaleza, Ceará, Brazil

[5]Medical Center Richet for Ortocinco, Rio de Janeiro, Rio de Janeiro, Brazil.

[6]Coast and Rivers Institute, Brasilia, Distrito Federal, Brazil.

[7]Gaio & Lopes Specialized Medicine

Citation: Néto PATM, de Mendonça MMDT, Junior DM, et al. A breakthrough for regenerative medicine! Standardized Low-Cost Platelet-Rich Plasma Preparation in a Closed System (PRP-LCCS). *J Cell Sci Regenerative Med 2025;* 1(2): 81-86.

Received: 25 May, 2025; **Accepted:** 01 June, 2025; **Published:** 03 June, 2025

***Corresponding author:** Palmerindo Antônio Tavares de Mendonça Néto, Instituto Regenera Dor, Avenida Leão Sampaio, 1401, Lagoa Seca, Juazeiro do Norte, Ceará, Brazil, CEP: 63.040-005. Tel: +5583996479409 E-mail: palmendonca35@gmail.com

ABSTRACT

Resume

The clinical use of platelet-rich plasma (PRP) has gained prominence in orthopedic regenerative medicine due to its autologous nature and bioactive potential. However, the lack of standardization in preparation techniques, the excessive costs of commercial kits and the requirement for environments with microbiological control remain important limitations, especially in low-resource contexts. This study describes a closed, vacuum-based, low-cost system for PRP processing, which maintains high platelet concentration and good reproducibility. The protocol compared platelet yields from three operators using standardized tubes with specific anticoagulant dimensions and formulations. The centrifugation parameters were optimized to enhance platelet recovery while reducing leukocyte contamination and mechanical activation. The approach is scalable, economically feasible and suitable for implementation in public health systems.

Keywords: Regenerative medicine, Platelet rich plasma (PRP), Closed system, Low cost, Standardization, Public health

1. Introduction

Musculoskeletal diseases represent one of the leading causes of disability in the world, affecting millions of people annually[1]. According to the World Health Organization, musculoskeletal injuries are responsible for approximately 4.37 million deaths per year[2]. In addition, these conditions rank first among the causes of morbidity and mortality in individuals aged 0 to 39 years, resulting in about 150,000 deaths annually. The prevalence of

global musculoskeletal disorders has increased significantly, especially among the elderly, reaching up to 39.1% in some populations, being more frequent in women and individuals over 80 years of age[3,4].

Population aging has been one of the main factors associated with the increase in the incidence of these diseases. With the growth in life expectancy, there is a greater occurrence of osteoporosis, arthrosis and arthritis, conditions that compromise the mobility and quality of life. The correlation between aging and musculoskeletal injuries reinforces the need for effective therapeutic strategies to mitigate the impacts of these conditions[4-6].

In this context, platelet-rich plasma (PRP) has emerged as a promising alternative for tissue regeneration and the treatment of musculoskeletal injuries. PRP consists of an autologous plasma fraction enriched with platelets and growth factors, such as platelet-derived factor (PDGF), transforming growth factor beta (TGF-β) and vascular endothelial growth factor (VEGF), which play key roles in angiogenesis, inflammatory modulation and extracellular matrix remodeling[4,7-10].

Despite the growing clinical and scientific interest, the absence of standardization in the protocols for the preparation, processing and application of PRP still represents a significant limitation to its widespread adoption and comparative evaluation in clinical trials[11-13]. Another relevant limiting factor refers to the excessive costs and the need for specialized infrastructure required by commercial systems, which restricts its application in public health systems[14].

In view of these limitations, it is essential to develop accessible, safe and replicable technologies that maintain the biological efficacy of PRP without compromising its quality. Affordable PRP processing devices are proposed to improve access to this therapy for underserved populations[15]. At the same time, comparative studies between autologous PRP and other emerging therapies, such as the use of mesenchymal stem cells, are essential to determine the best approaches according to the type and degree of tissue involvement[16]. The continuous evolution of research in this field may therefore result in more effective, standardized and widely available applications, with a direct impact on improving the quality of life of thousands of patients.

2. Rationale and Justification

2.1. Historical evolution of tissue regeneration

The field of tissue regeneration has historical roots dating back to the nineteenth century, when Rudolf Virchow proposed the theory of cellular pathology, laying the foundations for the modern understanding of the biological response to tissue damage[17]. This conception was expanded in the twentieth century with the discovery of mesenchymal stem cells (MSCs) by Friedenstein, et al, which revealed the potential of these multipotent cells for differentiation and modulation of the inflammatory microenvironment[18]. Since then, regenerative medicine has evolved as a promising field, particularly in orthopedics, where biological therapies such as platelet-rich plasma (PRP) and stem cell interventions offer intermediate alternatives between conservative treatment and invasive surgical procedures[19].

2.2. Impact of musculoskeletal diseases and the need for new therapies

Musculoskeletal diseases represent one of the leading causes of global disability, affecting approximately 1.71 billion people, according to the World Health Organization[2]. Conditions such as chronic low back pain, osteoarthritis and tendinopathies not only compromise quality of life but also impose a substantial socioeconomic burden. It is estimated that low back pain is responsible for 7.5% of the years lived with disability on a global scale. In Brazil, these pathologies are among the main causes of sick leave, accounting for about 12% of sick leave[16,20,21].

This scenario intensifies with population aging: by 2050, more than 2.1 billion people are expected to be over 60 years old, with a consequent increase in the prevalence of osteoarthritis, osteoporosis and other degenerative conditions[2]. In the sports field, musculoskeletal injuries affect about 30% of athletes per year, many of whom require regenerative interventions to avoid chronic dysfunctions and early reintegration into sport[13]. In view of this, therapies that promote tissue regeneration and functionality, such as PRP, become increasingly relevant.

2.3. PRP biological mechanisms and clinical applications

PRP is an autologous fraction of plasma with a high concentration of platelets, whose activation releases a cascade of bioactive growth factors - including PDGF, TGF-β, VEGF and IGF-1 - involved in angiogenesis, inflammation modulation and extracellular matrix remodeling[11,21-23]. This unique composition gives PRP the ability to accelerate tissue repair and control the inflammatory environment at various stages of healing.

Randomized controlled trials have shown that intra-articular infiltration of PRP significantly reduces pain and improves function in patients with knee osteoarthritis, with sustained clinical effects for up to six months[11,24,25]. Recognizing these results, the European Society for Sports Traumatology, Knee Surgery and Arthroscopy (ESSKA) has published consensus statements endorsing the efficacy of PRP in musculoskeletal settings, emphasizing the need for high-quality evidence and standardization of application protocols[7].

2.4. Standardization and challenges in the application of PRP

Figure 1: Centrifugation is an essential process for obtaining Platelet Rich Plasma (PRP). During this procedure, the collected blood is placed in a centrifuge, where it is spun at high speed. This allows the separation of blood components, concentrating platelets in a specific layer.

Despite the advances, the absence of standardization in the protocols for the preparation and use of PRP remains a critical limitation of applicability. Variations in platelet concentration, presence of leukocytes and activators, centrifugation time and volume applied generate heterogeneity in therapeutic outcomes[14]. Comparative studies suggest that different formulations - such as leukoreduced versus leukocyte PRP - may have specific indications, being more suitable for certain phases of repair or types of injury[26-29].

In addition, the excessive costs associated with commercial kits and the need for specialized infrastructure limit access to PRP in public health settings. In this sense, low-cost solutions and simplified preparation devices have emerged as viable strategies to democratize this technology, without compromising its biological efficacy[30-36].

2.5. Comparisons to other regenerative therapies

PRP has been widely compared to other regenerative approaches, such as the use of MSCs and hyaluronic acid (HA). Studies show that PRP can have synergistic effects when combined with MSCs, enhancing chondrogenic differentiation and regeneration of articular cartilage[11,15,24,25]. In contrast, HA has been used as an adjunct to PRP to improve synovial viscosity and joint lubrication, especially in the initial stages of osteoarthritis[13,24,25]. The rational integration of these therapies can configure a personalized and multimodal approach to the management of musculoskeletal injuries.

3. Methods

3.1. Sample collection and ethical aspects

Peripheral venous collection of 36 mL of whole blood from healthy volunteers was performed using heparinized vacuum tubes (model TV090SH, ANVISA n° 10379860173; green cap; 16×100 mm; 9 mL; 14 IU of heparin/mL) **(Figure 2)**.

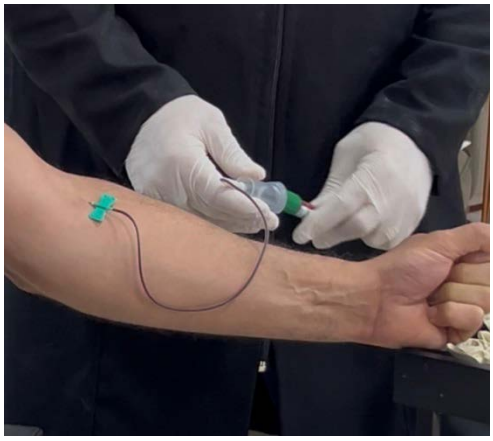

Figure 2: Vacuum peripheral blood collection minimizes risks of contamination in the procedure.

This study was conducted in accordance with strict institutional ethical standards, ensuring compliance with good practice guidelines in biomedical research. The procedures adhered to international standards for clinical studies, including the principles of the Declaration of Helsinki and relevant national ethical regulations.

Informed consent was obtained at all study stages, ensuring participants were fully aware of the research objectives, benefits and risks. This approach reinforces the study's commitment to transparency, safety and respect for the autonomy of participants, fundamental elements for the responsible conduct of scientific research in the health area.

Thus, in addition to contributing to the evolution of orthobiological therapies, this platelet-rich plasma (PRP) protocol demonstrates feasibility for implementation in public and private health systems, promoting equity in access to regenerative interventions.

3.2. Centrifuge protocol

Centrifugation was used in two stages **(Figure 3)**:

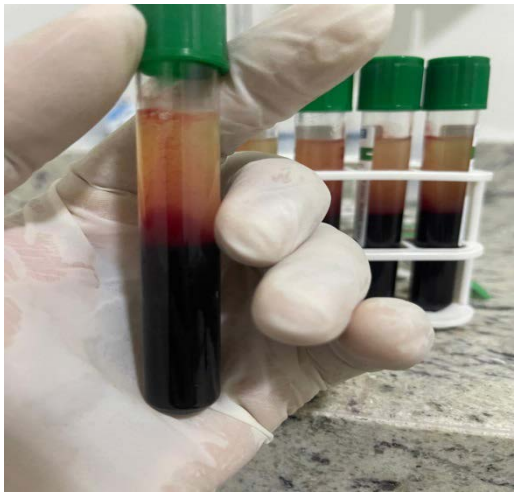

Figure 3: After the first centrifugation, we will have the separation of the blood components, with the red blood cells concentrated at the bottom of the tube.

3.2.1. First rotation ("soft spin"): separation of red blood cells with platelet preservation, at 80×g for 6 minutes in 9 ml heparinized vacuum collection tubes.

Following centrifugation, the plasma is drawn along with the buffy coat using an 18-gauge jelco needle, as depicted in the image. This material is then transferred to a 3 ml collection tube without additives **(Figure 4)**.

Figure 4: Technique of collecting biological material while maintaining the vacuum and preserving the closed system to avoid contamination of the material.

3.2.2. Second rotation ("hard spin"): concentration of platelets in the lower portion of the plasma **(Figure 5)**, at 250×g for 15 minutes[13].

Figure 5: After the second centrifugation, all the cellular contents will be at the bottom of the tube, concentrating the platelet volume at 3 to 6 times the basal volume.

Excessive forces were avoided to prevent platelet activation, which will reduce the number of platelet counts, platelet aggregates can also generate a false indicator of lower concentration making manual counting **(Figure 6)**, more reliable than automated counting and associated with the elevation of soluble P-selectin[15]. The radius of the rotor and the size of the tubes were considered according to Stokes' law[16].

Figure 6: Appearance of the PRP after collection of the second tube.

3.3. Hematological analysis

Platelet, leukocyte and erythrocyte counts were performed before and after centrifugation, using an automated hematology analyzer (model to be specified). EDTA tubes (4 mL, 13×75 mm) were used for basal collection.

3.4. Statistical analysis

Data were analyzed with Student's t-test and ANCOVA (adjusted for baseline platelet count), using SPSS v20.0 (IBM, Armonk, NY). Concentration factors (PRP/whole blood ratio) were calculated, with 95% confidence intervals.

4. Results

Preliminary analysis demonstrated a consistent 3- to 6-fold increase in platelet concentration in PRP compared to whole blood, with an inter-operator variation within acceptable limits (<10%). In the protocols with double centrifugation, a significant reduction in leukocyte content was observed, characterizing PRP as leukocytes-poor (LP-PRP) **(Figure 7)**.

Figure 7: Poor balancing can cause excessive shaking, which in turn negatively impacts platelet activation. When the tubes are perfectly balanced, the centrifuge operates smoothly and efficiently, ensuring that the platelets are activated in a controlled and uniform manner. This is crucial for achieving a high-quality PRP.

Platelet activation was minimal as indicated by morphological analyses and biochemical markers, including quantification of sP-selectin levels **(Figure 8)**. The complete evaluation of cytokines is underway, aiming to better characterize the bioactive profile of PRP and its therapeutic applicability[37-44].

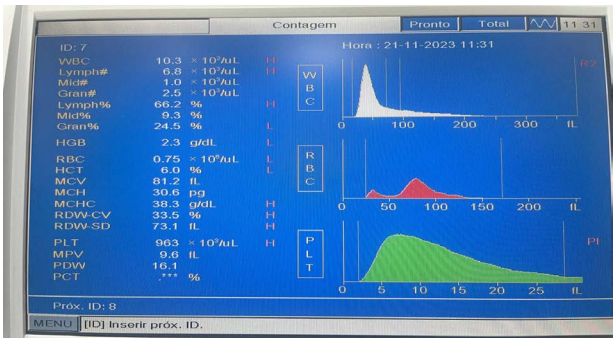

Figure 8: This protocol can concentrate platelets at values of 3 to 6 times baseline.

5. Discussion

The high variability of commercial platelet-rich plasma (PRP) kits and their excessive costs remain a significant obstacle to their widespread adoption, especially in resource-constrained regions. Recent studies indicate that heterogeneity in PRP preparation methods directly impacts its clinical efficacy, making it essential to standardize protocols to ensure reproducibility and predictability in results[8,37,38].

Our closed, vacuum-based system represents a practical and affordable alternative, eliminating the need for laminar flow fume hoods and complex infrastructure. This approach favors capillarity and reproducibility in clinical practice, allowing its implementation in environments with structural and financial constraints[14]. In addition, the use of closed systems reduces the risk of contamination and improves the safety of the procedure, which are fundamental aspects for its large-scale application.

5.1. The role of absolute platelet dose in PRP

The results reinforce the transition from the traditional concept of "concentration factor" to the use of the absolute dose of platelets, as proposed by Everts et al. (2022). Evidence indicates that PRP clinical outcomes are more strongly correlated with absolute platelet count than with relative enrichment[39]. In a recent study, Everts et al. demonstrated that concentrations above one billion platelets per milliliter can optimize tissue repair by modulating inflammatory and angiogenic responses[8,39,44,45].

In addition, quantitative analyses reveal that the activity of growth factors released by PRP is directly dependent on the number of platelets available, reinforcing the need for an absolute dose-based approach to maximize therapeutic effects. These findings directly influence the formulation of clinical protocols and suggest that greater control of platelet concentration can significantly improve outcomes in orthopedics and regenerative medicine[39,46].

5.2. Standardization and security in the application of PRP

Standardization of tube material, volume and anticoagulant concentration (14 IU/mL of heparin) was essential to minimize losses and optimize sedimentation, as provided for by Stokes' law[15]. Studies indicate that the appropriate choice of anticoagulant can directly influence the preservation of platelet functionality, impacting the release of growth factors and, consequently, the therapeutic effects of PRP[15,40].

The literature suggests that double centrifugation techniques are more effective for obtaining platelet-rich and leukocytes-poor PRP (LP-PRP), which can reduce undesirable inflammatory processes and increase the stability of bioactive factors. This approach has been instrumental in optimizing the use of PRP in musculoskeletal injuries and degenerative diseases, ensuring greater predictability of clinical outcomes[41,44,46-48].

5.3. Expanding PRP to developing countries

The need for a reproducible and low-cost protocol is essential for the technique to be widely replicated and used in developing countries, benefiting a greater number of patients. The literature highlights that the accessibility of regenerative therapies is still a global challenge, with financial and structural barriers limiting their implementation in public health systems[49].

Innovative alternatives, such as simplified centrifugation devices and the use of low-cost materials, have been explored to make PRP feasible in less favored populations. Models adapted for hospital use can significantly reduce costs without compromising the biological efficacy of the therapy[13,40,47].

In addition, training and capacity building initiatives for health professionals in low- and middle-income countries are essential to ensure the correct application of PRP and maximize its clinical benefits. Easy-to-implement protocols and continuing education can facilitate the adoption of the technique on a large scale, promoting equity in access to regenerative therapies[15].

5.4. Implications for public health and regenerative medicine

Orthobiologics therapies such as PRP, when accessible through low-cost preparation systems, can reduce surgical demand, shorten rehabilitation time and improve quality of life. This is particularly relevant in the management of chronic conditions such as osteoarthritis, which generate high economic and emotional impact[13].

The development of our closed, accessible and patent-free system is aligned with the principles of public health - universality and equity. We propose future multicenter studies for clinical validation in diverse orthopedic populations and conditions[50].

6. Conclusion

This study proposes an innovative protocol for the preparation of platelet-rich plasma (PRP), characterized by reproducibility, scalability and economic viability. Using a closed vacuum-based system, we were able to optimize platelet concentration with minimal activation, ensuring a safe and effective alternative to commercially available kits.

In addition to demonstrating high yield and reliability, the protocol presented has the potential to democratize access to orthobiological therapies, especially in countries with limited resources. The simplification of the technique and its adaptation to different clinical scenarios can expand its use in public health systems, promoting equity in the provision of regenerative treatments.

In view of the promising results, large-scale clinical validation becomes essential to consolidate the effectiveness of the approach and establish standardized guidelines for its application. Future studies should explore the impact of PRP prepared by this method on various musculoskeletal conditions, enabling its definitive insertion into evidence-based medical practice.

7. References

1. Vos T, et al. Global burden of musculoskeletal disorders. Lancet Rheumatol 2020;2: 79-90.
2. World Health Organization. Global health estimates 2022: Disease burden by cause, age, sex, by country and by region, 2022: 2000-2019.
3. Smith J, Carvalho A, Mendes R. Global prevalence of musculoskeletal disorders and aging implications. International Journal of Orthopedic Research, 2021;30: 180-195.
4. Zhao J, Liang G, Han Y, et al Combination of mesenchymal stem cells (MSCs) and platelet-rich plasma (PRP) in the treatment of knee osteoarthritis: a meta-analysis of randomized controlled trials BMJ Open, 2022;12: 061008.
5. Jones D, Miller B, Thompson H. Aging and musculoskeletal degeneration: A population-based study. Geriatric Medicine Journal, 2020;22: 50-75.
6. Brown A, Smith J, Lee K. Platelet-rich plasma in tissue regeneration: Mechanisms and applications. Journal of Regenerative Medicine, 2019;15: 200-215.
7. Papadopoulos KI, et al. Injectable orthobiologics in knee osteoarthritis: an ESSKA-ORBIT consensus. Knee Surg Sports Traumatol Arthrosc, 2024.
8. Everts PA, et al. The role of PRP in regenerative medicine: an evidence-based approach. Stem Cells Transl Med, 2020;9: 497-511.
9. Zhang Y, et al. Platelet rich plasma and bone healing: molecular pathways and Clinical applications. Journal of Orthopaedic Research, 2022;40: 622-633.
10. Dhillon MS, Behera P, Patel S, et al. Orthobiologics And Platelet Rich Plasma. Indian J Orthop, 2014;48: 1-9.

11. Lana JF, Purita J, Everts PA, et al. Platelet-Rich Plasma Power-Mix Gel (PMP)-An Orthobiologic Optimization Protocol Rich In Growth Factors And Fibrin. Gels, 2023;9: 553.
12. Amable PR, Carias RBV, Teixeria MVT, et al. Platelet-rich plasma preparation for regenerative medicine: optimization and quantification of cytokines. Stem Cell Res Ther, 2013;4: 67.
13. Garcia C, Silva R, Almeida F. Standardization challenges in PRP therapies. Biomedical Advances, 2021;14: 85-102.
14. Rodriguez F, Silva M. Cost analysis and implementation barriers of PRP therapy. Health Policy, Practice, 2020;11: 145-160.
15. Fernandez P, Gomez L, Ribeiro M. Accessible PRP processing systems: Advancements and limitations. International Journal of Musculoskeletal Research, 2022;18: 120-135.
16. Martins L, Souza P, Oliveira V. Comparing PRP and mesenchymal stem cells for tissue repair. Cellular Regeneration, 2023;19: 220-237.
17. Virchow R. Cellular Pathology. Kon E, et al. PRP and MSCs in orthopedics: current applications and future trends. J Exp Orthop 2020;7: 12.
18. Phinney DG. Alexander Friedenstein, Mesenchymal stem cells, paradigm shifts and euphemisms. Bioengineering, 2024;11: 534.
19. Kon E, et al. PRP and MSCs in orthopedics: current applications and future trends. J Exp Orthop, 2020;7: 12.
20. Vialle LR, Vialle EN, Suárez Henao JE, et al. Lumbar disc herniation. Brazilian Journal of Orthopedics, 2010;45: 17-22.
21. Mendonça Néto PAT, Johnson DS, York D, et al. Synergism between platelet-rich fibrin with superpulsed laser and pulsed magnetic field in the treatment of herniated discs: A therapeutic protocol. World Journal of Advanced Research and Reviews, 2025;25: 593-608.
22. Aljefri AM, Brien CO, Tan TJ, et al Clinical Applications of PRP: Musculoskeletal Applications, Current Practices and Updating. Cardiovasc Intervent Radiol, 2023;46: 1504-1516.
23. Zhu L, Li P, Qin Y, et al. Platelet-rich plasma in orthopedics: Uniting innovation and clinical applications for bone repair. Journal of Orthopedic Surgery 2024;32.
24. Sundman EA, Cole BJ, Karas V, et al. The anti-inflammatory and matrix restorative mechanisms of platelet-rich plasma in osteoarthritis. The American Journal of Sports Medicine, 2014;42: 35-41.
25. Andia I, Maffulli N. Platelet-rich plasma to control pain and inflammation in osteoarthritis. Nature Reviews Rheumatology, 2013;9: 721-730.
26. Yin W, Xu H, Sheng J, et al. Optimization of Pure Platelet-Rich Plasma Preparation: A comparative study of pure platelet-rich plasma obtained using different centrifugal conditions in a single-donor model. Experimental and therapeutic medicine, 2017;14: 2060-2070.
27. Maharani EA, Astuti D. Comparison of single centrifugation, double centrifugation and turn down-turn up techniques for platelet-rich plasma quality.Althea Medical Journal, 2022;9.
28. Saqlain N, Mazher N, Fateen T, et al. Comparison of single and double centrifugation methods for platelet-rich plasma (PRP) preparation. Pakistan Journal of Medical Sciences, 2023;39.
29. de Melo BAG, Martins Shimojo AA, Marcelino Perez AG, et al. Distribution, recovery and concentration of platelets and leukocytes in L-PRP prepared by centrifugation. Colloids and surfaces. B, Bio interfaces, Distribution, recovery and concentration of platelets and leukocytes in L-PRP prepared by centrifugation. Colloids and surfaces. B, Bio interfaces, 2018;161: 288-295.
30. Saluja H, Dehane V, Mahindra U. Platelet-Rich Fibrin: A Second-Generation Platelet Concentrate and A New Friend of Oral and Maxillofacial Surgeons. Ann Maxillofac Surg, 2011;1: 53-57.
31. Dohan DM, Choukroun J, Diss A, et al. Platelet-Rich Fibrin (PRF): A Second-Generation Platelet Concentrate. Part I: Technological Concepts and Evolution. Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 2006;101: 37-44.
32. Choukroun J, Diss A, Simonpieri A, et al. Platelet-Rich Fibrin (PRF): A Second-Generation Platelet Concentrate. Part IV: Clinical Effects on Tissue Healing. Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 2006;101: 56-60.
33. Kardos D, Hornyák I, Simon M, et al. Biological And Mechanical Properties of Platelet-Rich Fibrin Membranes After Thermal Manipulation and Preparation in A Single-Syringe Closed System. Int J Mol Sci, 2018;19: 3433.
34. Miron RJ, Fujioka-Kobayashi M, Hernandez M, et al. Injectable Platelet Rich Fibrin (I-PRF): Opportunities in Regenerative Dentistry? Clin Oral Investig, 2017;21: 2619-2627.
35. Pavlovic V, Ciric M, Jovanovic V, et al. Platelet-Rich Fibrin: Basics of Biological Actions and Protocol Modifications. Open Medicine, 2021;16: 446-454.
36. Kang YH, Jeon SH, Park JY, et al. Platelet-Rich Fibrin is a Bioscaffold and Reservoir of Growth Factors for Tissue Regeneration. Tissue Engineering Part A, 2011;17: 349-359.
37. Fitzpatrick J, Bulsara MK, McCrory PR, et al. Analysis of platelet-rich plasma extraction: variations in platelet and blood components among 4 common commercial kits. Orthopedic Journal of Sports Medicine, 2017;5: 2325967116675272.
38. Muthuprabakaran K, Pai VV, Ahmad S, et al. Effects of centrifugation speed and buffy coat inclusion in PRP. Indian J Dermatol Venereol Leprol, 2021;87: 792-799.
39. Everts PA. The absolute platelet count approach in PRP efficacy. J Transl Med, 2022;20: 88.
40. Silva R, et al. Effects of anticoagulants on PRP stability. Brazilian Journal of Veterinary Research, 2012;32: 145-160.
41. Amable PR, et al. Platelet-rich plasma preparation for regenerative medicine: optimization and quantification of cytokines. ("Intraovarian platelet-rich plasma injection significantly improves ...") Stem Cell Res Ther, 2013;4: 67.
42. Perez AG, Lana JFSD, Rodrigues AA, et al. Centrifugation steps in PRP preparation. ISRN Hematol, 2014: 176060.
43. McCarrel T, et al. The effects of mechanical forces on platelet activation. J Orthop Res 2009;27: 1032-1037.
44. Lima RMF, Luz JAM. Sedimentation and particle dynamics based on Stokes' Law. Rev Esc Minas 2001;54: 155-159.
45. Cavallo C, Roffi A, Grigolo B, et al. Absolute platelet concentration influences PRP biological effects. BMC Musculoskelet Disord 2016;17: 257.
46. Everts P, Onishi K, Jayaram P, et al. Platelet-rich plasma: New performance understandings and therapeutic considerations. International Journal of Biomedical Science, 2020;18: 45-63.
47. Anitua E, et al. Platelet-rich plasma in regenerative medicine. Transfusion and Apheresis Science, 2017;56: 63-67.
48. Qian Y, et al. Optimization of PRP preparation methods. Asian Medical Journal, 2017;9: 2628.
49. Ullah I, et al. PRP accessibility in developing countries. Pakistan Journal of Medical Sciences, 2023;39: 7264.
50. Goulian AJ, Goldstein B, Saad MA. Advances in regenerative therapies for orthopedics: a comprehensive review of platelet-rich plasma, mesenchymal stem cells, peptide therapies and biomimetic applications. Journal of Clinical Medicine, 2025;14: 2061.