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ADVANCED PLATELET-RICH FIBRIN (A-PRF+): BIOLOGICAL RATIONALE, PREPARATION PROTOCOLS, AND CLINICAL APPLICATIONS IN ORAL REGENERATIVE THERAPY

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Abstract

Advanced platelet-rich fibrin (A-PRF+) is a third-generation autologous platelet concentrate developed through the low-speed centrifugation concept, aiming to enhance the biological performance of platelet-derived biomaterials in oral regenerative therapy. By reducing relative centrifugal force during preparation, A-PRF+ achieves a more homogeneous incorporation of platelets and leukocytes within a loosely organized fibrin matrix, resulting in sustained release of growth factors and active immunomodulation. This review synthesizes current evidence regarding the biological rationale, preparation protocols, growth factor dynamics, and clinical applications of A-PRF+ in periodontal, implant-related, and oral surgical procedures. Available data indicate that A-PRF+ promotes angiogenesis, fibroblast migration, and osteogenic activity while providing antimicrobial and anti-inflammatory benefits within the oral environment. However, variability in preparation protocols, patient-related modifiers such as smoking, and methodological limitations of existing studies continue to challenge clinical standardization. Future research should focus on protocol harmonization, personalized regenerative strategies, and long-term clinical outcomes to better define the role of A-PRF+ in modern oral regenerative therapy.
Keywords: A-PRF+, platelet-rich fibrin, oral regeneration, growth factors, low-speed centrifugation

Conceptual and Biological Rationale of A-PRF+

Autologous platelet concentrates have progressively evolved as biologically driven tools in oral regenerative therapy, aiming to enhance wound healing and tissue regeneration through endogenous growth factors and cellular mediators. Among these, platelet-rich fibrin (PRF), introduced by Choukroun as a second-generation platelet concentrate, represented a major conceptual shift by eliminating anticoagulants and bovine thrombin, thus allowing the formation of a natural fibrin scaffold enriched with platelets and leukocytes [11]. This biological matrix provides not only mechanical stability but also a sustained release of growth factors, creating a microenvironment favorable for tissue repair [1].

The initial clinical success of leukocyte- and platelet-rich fibrin (L-PRF) prompted further investigation into how centrifugation parameters influence the biological quality of PRF matrices. Experimental and clinical evidence demonstrated that high relative centrifugal forces (RCF) lead to excessive cellular stratification, limiting the entrapment of leukocytes and progenitor cells within the fibrin network [2]. This observation formed the foundation of the Low Speed Centrifugation Concept (LSCC), which proposes that reducing centrifugation speed and force results in improved cellular preservation and enhanced biological activity of PRF matrices [2].

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Advanced platelet-rich fibrin (A-PRF) and its optimized variant, A-PRF+, were developed directly from the LSCC paradigm. By applying lower RCF values over adjusted centrifugation times, A-PRF+ demonstrates a significantly higher concentration of leukocytes, particularly neutrophils and monocytes, distributed more homogeneously throughout the fibrin clot [2]. These cells play a critical role in the orchestration of tissue regeneration by modulating inflammation, promoting angiogenesis, and regulating fibroblast and osteoblast activity [3]. Unlike earlier PRF formulations, A-PRF+ is therefore not merely a platelet-derived growth factor reservoir, but a dynamic immuno-regenerative construct.

The biological rationale of A-PRF+ is tightly linked to the multifunctional role of leukocytes in wound healing. Leukocytes embedded within the fibrin scaffold contribute to antimicrobial defense, an aspect particularly relevant in the oral cavity, where surgical sites are constantly exposed to microbial challenges [3]. Moreover, monocytes and macrophages derived from A-PRF+ participate in the transition from the inflammatory to the proliferative phase of healing, facilitating extracellular matrix deposition and neovascularization [5]. This controlled inflammatory milieu distinguishes A-PRF+ from PRP-based systems, which often induce short-lived growth factor bursts without sustained cellular support [5].

From a structural standpoint, the fibrin architecture of A-PRF+ exhibits a looser, more porous network compared to L-PRF, allowing greater cell migration and nutrient diffusion [4]. This three-dimensional fibrin scaffold functions as a provisional extracellular matrix, guiding cell adhesion and proliferation while gradually releasing bioactive molecules. Studies quantifying growth factor release have consistently shown that A-PRF+ provides prolonged and higher cumulative levels of key mediators such as transforming growth factor-beta (TGF-β), platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF), which are essential for soft tissue maturation and bone regeneration [8].

The superiority of A-PRF+ in biological terms is further supported by in vitro wound healing models. Periodontal fibroblasts exposed to A-PRF+ demonstrate enhanced migration, proliferation, and collagen synthesis compared to conventional PRF preparations [9]. These cellular responses are clinically relevant, as fibroblast activity is a determinant factor in gingival wound closure, connective tissue stability, and esthetic outcomes following periodontal and mucogingival surgery. Additionally, experimental in vivo models have confirmed increased vascularization and cellular infiltration when low-speed centrifugation protocols are applied, reinforcing the translational value of A-PRF+ [10].

Another critical biological aspect underpinning A-PRF+ is the influence of centrifugation physics on platelet integrity and function. Variations in centrifugal force affect platelet activation status and aggregation behavior, which in turn modulate growth factor release kinetics [7]. Reduced centrifugation speeds preserve platelet morphology and functionality, favoring a gradual release profile rather than an abrupt degranulation. This controlled delivery aligns more closely with physiological wound healing processes and reduces the risk of premature matrix degradation [6].

Furthermore, comparative analyses between PRP, PRF, and advanced PRF formulations consistently indicate that A-PRF+ offers a more favorable balance between cellular content, scaffold stability, and biological signaling [14]. The enhanced biocompatibility and sustained growth factor availability observed with A-PRF+ support its expanding role in regenerative protocols where predictable tissue integration is required. Importantly, the introduction of horizontal centrifugation systems has further refined the biological consistency of A-PRF+ by improving cell distribution within the clot, addressing one of the major limitations of early PRF techniques [13].

In summary, the conceptual and biological rationale of A-PRF+ is grounded in a paradigm shift from platelet concentration alone toward a cell-driven, immunologically active regenerative scaffold. By integrating optimized centrifugation physics with an understanding of

wound healing biology, A-PRF+ represents an evolution of autologous biomaterials tailored to the complex regenerative demands of oral and maxillofacial therapy [15].

Preparation Protocols and Technical Parameters of A-PRF+

The biological performance of advanced platelet-rich fibrin (A-PRF+) is intrinsically dependent on the technical parameters applied during its preparation, rendering protocol standardization a critical determinant of clinical predictability. Unlike earlier platelet concentrates, A-PRF+ is not defined solely by its autologous origin, but by a carefully calibrated centrifugation strategy designed to preserve cellular viability, optimize fibrin architecture, and ensure sustained bioactive molecule release [18]. Consequently, preparation protocols must be interpreted as biologically active variables rather than simple laboratory steps.

A central parameter in A-PRF+ preparation is the relative centrifugal force (RCF), which directly influences cellular stratification within the clot. High centrifugal forces promote rapid sedimentation of erythrocytes and platelets, but simultaneously displace leukocytes toward the distal portion of the tube, reducing their incorporation into the fibrin matrix [21]. In contrast, reduced RCF values—characteristic of A-PRF+ protocols—facilitate a more homogeneous distribution of leukocytes and platelets throughout the fibrin scaffold, enhancing its regenerative potential [22]. This principle is consistent with experimental evidence demonstrating that subtle changes in g-force can significantly alter the biological composition of platelet concentrates [21].

The standard A-PRF+ protocol typically involves centrifugation at approximately 1300 rpm for 8 minutes, although absolute values vary depending on rotor radius and centrifuge design [22]. This underscores the importance of calculating RCF rather than relying solely on revolutions per minute (rpm), as identical rpm values may generate markedly different centrifugal forces across devices. Failure to account for these variations contributes to protocol heterogeneity and limits inter-study comparability [19]. Therefore, reporting centrifuge specifications and calculated RCF values is essential for methodological transparency and reproducibility.

Another technical consideration is the orientation of centrifugation. Vertical centrifugation, traditionally used in PRF preparation, has been associated with uneven cell layering and limited control over clot architecture. More recent approaches employing horizontal centrifugation have demonstrated superior cellular distribution and improved platelet and leukocyte entrapment within the fibrin matrix [13]. Horizontal systems minimize radial acceleration gradients, thereby reducing cellular displacement and enhancing biological consistency. This technical refinement has been shown to increase the concentration of growth factors and inflammatory cells in the upper and central portions of the A-PRF+ membrane, which are most frequently utilized in clinical applications [23].

Blood collection and handling protocols further influence the quality of A-PRF+. Immediate centrifugation following venipuncture is mandatory, as delays promote premature coagulation and compromise fibrin polymerization dynamics [20]. The use of glass-coated or silica-free tubes is also critical, as surface chemistry affects clot initiation and fibrin organization. Inappropriate tube selection may lead to irregular clot formation or reduced mechanical stability of the membrane, directly impacting its surgical handling properties [18].

Patient-related variables introduce additional complexity into A-PRF+ preparation. Systemic and behavioral factors, particularly cigarette smoking, have been shown to alter platelet function, leukocyte activity, and inflammatory responses [16,17]. Smoking-induced vasoconstriction and oxidative stress may impair platelet activation and growth factor release, potentially diminishing the biological efficacy of A-PRF+ membranes. These effects highlight the necessity of contextualizing preparation protocols within patient-specific biological conditions, rather than assuming uniform outcomes across populations.

From a translational perspective, the preparation of A-PRF+ represents a convergence of centrifugation physics, hematological biology, and clinical pragmatism. While numerous protocols have been proposed, the lack of universal standardization remains a limitation in both clinical practice and research [19]. Nonetheless, emerging classification systems for platelet concentrates aim to harmonize terminology and preparation criteria, facilitating more reliable comparisons and evidence synthesis [18].

In summary, the preparation protocols of A-PRF+ are not merely technical procedures but biologically determinant processes that directly modulate regenerative outcomes. Optimization of centrifugal force, centrifugation geometry, blood handling, and patient-related variables is essential to fully exploit the therapeutic potential of A-PRF+ in oral regenerative therapy [23].

Table 1. Key Preparation Parameters and Technical Variables Influencing A-PRF+ Quality

Parameter	Description	Biological / Clinical Impact
Relative Centrifugal Force (RCF)	Reduced g-force compared to L-PRF	Enhances leukocyte retention and homogeneous cell distribution
Centrifugation Time	Shortened duration (≈8 minutes)	Preserves platelet integrity and limits cellular stratification
Centrifugation Speed (RPM)	Device-dependent; RCF-calculated	Improves protocol reproducibility
Rotor Geometry	Vertical vs. horizontal centrifugation	Horizontal systems improve cell accumulation
Blood Handling Time	Immediate centrifugation post-collection	Prevents premature coagulation
Collection Tubes	Glass-coated or silica-free	Ensures stable fibrin architecture
Patient-Related Factors	Smoking, inflammatory status	May alter platelet activation and growth factor release

Table 1 summarizes the critical technical and procedural variables involved in A-PRF+ preparation. Optimization of centrifugation physics and blood handling is essential to ensure biological consistency and predictable regenerative performance.

Growth Factor Release and Cellular Interactions in A-PRF+

The regenerative efficacy of advanced platelet-rich fibrin (A-PRF+) is primarily governed by its ability to ensure a sustained release of growth factors while simultaneously acting as a biologically active scaffold that orchestrates cellular interactions involved in wound healing and tissue regeneration. Unlike platelet-rich plasma (PRP), which is characterized by an abrupt and short-lived release of bioactive mediators, A-PRF+ exhibits a prolonged and gradual delivery profile that more closely resembles physiological healing dynamics [2,8,14].

Quantitative investigations have consistently demonstrated that A-PRF+ releases higher cumulative levels of key growth factors, including transforming growth factor-beta (TGF-β), platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF), over extended time intervals compared to L-PRF and PRP [8,14,21]. This sustained release is directly attributable to the low-speed centrifugation concept, which preserves platelet integrity and facilitates their homogeneous incorporation within a loosely organized fibrin matrix [2,14]. TGF-β plays a pivotal role in fibroblast proliferation, collagen synthesis, and extracellular matrix remodeling, while PDGF exerts potent chemotactic and mitogenic effects on mesenchymal cells, including osteoblasts and periodontal ligament fibroblasts. VEGF is

essential for angiogenesis, supporting neovascularization during the early phases of tissue regeneration [8,14].

Beyond its growth factor reservoir function, A-PRF+ is distinguished by its enriched leukocyte content, which confers important immunomodulatory properties. Monocytes and neutrophils embedded within the fibrin scaffold actively participate in the regulation of inflammation by secreting cytokines, proteolytic enzymes, and signaling molecules that coordinate tissue turnover and repair [5,22]. Monocyte-derived macrophages, in particular, are central to the transition from the inflammatory to the proliferative phase of healing, adopting pro-regenerative phenotypes that support angiogenesis and osteogenesis [22]. This controlled inflammatory response represents a fundamental biological advantage of A-PRF+ over platelet concentrates devoid of leukocytic components.

At the cellular level, A-PRF+ has been shown to significantly enhance fibroblast migration and proliferation in in vitro wound healing models. Periodontal fibroblasts exposed to A-PRF+ demonstrate accelerated wound closure and increased collagen production, findings that are directly relevant to clinical outcomes in mucogingival and periodontal surgery [9]. Similarly, osteoblastic cells respond favorably to A-PRF+-derived signaling, exhibiting increased proliferation and upregulation of osteogenic markers, thereby supporting its adjunctive use in bone regenerative procedures [18,19].

Angiogenesis represents another critical biological mechanism mediated by A-PRF+. The fibrin network functions as a provisional extracellular matrix that facilitates endothelial cell adhesion and capillary sprouting. These processes are synergistically driven by VEGF gradients and leukocyte-derived mediators, resulting in enhanced vascular infiltration and improved nutrient diffusion within regenerating tissues [10,14]. In vivo studies have corroborated these findings, demonstrating increased vascular density and cellular infiltration when low-speed centrifugation protocols are applied [10].

Table 2. Major Growth Factors and Cellular Interactions Mediated by A-PRF+

Biological Component	Primary Source in A-PRF+	Main Regenerative Function
TGF-β	Platelets, leukocytes	Fibroblast proliferation and extracellular matrix remodeling
PDGF	Platelets	Chemotaxis and proliferation of mesenchymal cells
VEGF	Platelets, monocytes	Angiogenesis and neovascularization
Monocytes	Leukocyte-rich fibrin matrix	Immunomodulation and tissue remodeling
Neutrophils	Leukocyte-rich fibrin matrix	Antimicrobial defense and inflammatory regulation
Fibrin Matrix	Polymerized fibrin network	Provisional extracellular scaffold
Endothelial Cells (target)	Stimulated by VEGF and cytokines	Capillary sprouting and vascular infiltration

Table 2 outlines the principal growth factors and cellular components involved in A-PRF+-mediated regeneration, highlighting their synergistic roles in angiogenesis, immunomodulation, and tissue repair.

A-PRF+ integrates sustained growth factor release with dynamic cellular interactions, encompassing immunomodulation, angiogenesis, and tissue-specific cell activation. This

multifaceted biological profile underpins its growing relevance as a predictable and physiologically aligned biomaterial in oral regenerative therapy [8,14,22].

Clinical Applications in Oral and Maxillofacial Regenerative Therapy

Advanced platelet-rich fibrin (A-PRF+) has gained increasing clinical relevance in oral and maxillofacial regenerative therapy due to its favorable biological profile, autologous nature, and versatility across a wide range of surgical and regenerative indications. Its capacity to enhance both soft and hard tissue healing positions A-PRF+ as a valuable adjunct in procedures requiring predictable wound stabilization and accelerated regeneration [1,11,18].

In periodontal therapy, A-PRF+ has been extensively applied in the treatment of intrabony defects, either alone or in combination with guided tissue regeneration (GTR) techniques. Clinical trials have demonstrated significant improvements in probing depth reduction, clinical attachment level gain, and radiographic bone fill when A-PRF+ is used as an adjunct to conventional regenerative protocols [4,8,15,23].

These outcomes are attributed to its sustained release of growth factors and its ability to stabilize the blood clot within periodontal defects, thereby facilitating cellular migration, angiogenesis, and osteogenic differentiation [8,23]. Compared with traditional membranes or biologic agents, A-PRF+ offers a biologically active alternative without the risk of immunogenic reactions.

Mucogingival and periodontal plastic surgery represent another important field of application for A-PRF+. Its use at free gingival graft donor sites, coronally advanced flap procedures, and root coverage techniques has been associated with accelerated epithelialization, reduced postoperative discomfort, and improved soft tissue quality [1,9]. The enhanced fibroblast activity and collagen deposition induced by A-PRF+ contribute to improved tissue thickness, color match, and long-term stability, which are critical determinants of esthetic success in periodontal surgery [9].

In implant dentistry, A-PRF+ has been employed in socket preservation, ridge augmentation, and sinus floor elevation procedures, frequently in combination with autogenous or xenogeneic bone grafts. The fibrin matrix of A-PRF+ acts as a biological binder, improving graft cohesion and handling, while its growth factor content promotes early vascularization and osteogenesis [18,21].

Additionally, the application of A-PRF+ around dental implants has been associated with improved soft tissue healing and reduced early inflammatory responses, potentially contributing to enhanced peri-implant tissue stability [21].

In oral and maxillofacial surgery, A-PRF+ has demonstrated utility in the management of extraction sockets, cystic defects, and surgical wounds. Its leukocyte-rich composition confers antimicrobial and immunomodulatory effects, which are particularly advantageous in the microbially challenged oral environment [3,5]. Enhanced hemostasis, reduced postoperative edema, and improved patient-reported outcomes further support its integration into routine surgical practice [5].

Despite these favorable clinical outcomes, the effectiveness of A-PRF+ may be influenced by patient-related factors such as smoking, which negatively affects platelet function, angiogenesis, and inflammatory regulation [16,17].

Therefore, careful patient selection and protocol adaptation are essential to maximize clinical benefits. Overall, current evidence supports A-PRF+ as a versatile and biologically

effective adjunct that bridges experimental regenerative principles with practical clinical application in oral and maxillofacial therapy [18,23].

Limitations, Patient-Related Factors, and Future Perspectives

Despite the growing body of evidence supporting the regenerative benefits of advanced platelet-rich fibrin (A-PRF+), several biological, technical, and clinical limitations must be acknowledged to ensure a balanced interpretation of its therapeutic potential. One of the principal challenges associated with A-PRF+ is the lack of universal standardization regarding preparation protocols. Variations in centrifugation devices, rotor geometry, relative centrifugal force (RCF), and centrifugation time result in heterogeneity of the final product, limiting the reproducibility and comparability of clinical outcomes across studies [18,19].

Patient-related biological variability represents another significant factor influencing the efficacy of A-PRF+. Systemic conditions, inflammatory status, and behavioral habits, particularly cigarette smoking, have been shown to alter platelet function, leukocyte activity, and growth factor release. Smoking-induced vasoconstriction, oxidative stress, and impaired immune responses can negatively affect angiogenesis and tissue remodeling, potentially reducing the regenerative performance of A-PRF+ membranes [16,17]. These findings underscore the importance of individualized treatment planning and cautious interpretation of outcomes in compromised patient populations.

From a methodological standpoint, many clinical studies evaluating A-PRF+ are characterized by short follow-up periods, small sample sizes, and heterogeneous study designs. This limits the strength of evidence regarding long-term stability and comparative effectiveness relative to other regenerative modalities [15,18]. Additionally, A-PRF+ is often used as an adjunct rather than as a standalone intervention, making it difficult to isolate its specific contribution to clinical outcomes. Well-designed randomized controlled trials with standardized protocols and long-term evaluation are therefore essential to establish robust clinical guidelines.

Technical limitations related to handling and application should also be considered. Although A-PRF+ exhibits improved mechanical properties compared to earlier PRF formulations, its resorption rate and structural stability remain dependent on defect morphology and surgical technique. In large or non-contained defects, A-PRF+ alone may be insufficient to maintain space and may require combination with bone grafts or barrier membranes to achieve optimal regenerative outcomes [18,21].

Looking toward future perspectives, increasing attention is being directed toward the personalization of A-PRF+ protocols. Adjusting centrifugation parameters based on patient-specific hematological profiles may enhance cellular yield and biological activity, aligning regenerative strategies with precision medicine principles [2,14]. Furthermore, emerging approaches involving horizontal centrifugation systems and refined classification schemes for platelet concentrates aim to improve standardization and reproducibility [13,18].

Innovative research is also exploring the synergistic use of A-PRF+ with biomaterials, stem cells, and drug delivery systems to further enhance regenerative outcomes. The integration of A-PRF+ into tissue engineering frameworks, including scaffold functionalization and controlled release systems, represents a promising avenue for expanding its clinical indications [6,19].

Conclusions

Advanced platelet-rich fibrin (A-PRF+) represents a biologically refined evolution of autologous platelet concentrates, integrating optimized centrifugation physics with the physiological principles of wound healing. Through enhanced leukocyte incorporation, a porous fibrin architecture, and sustained growth factor release, A-PRF+ functions as an immuno-regenerative scaffold capable of supporting angiogenesis, fibroblast activity, and osteogenesis, thereby aligning closely with the complex biological demands of oral and maxillofacial tissue regeneration.

Despite its demonstrated clinical versatility and favorable biological profile, the therapeutic efficacy of A-PRF+ remains influenced by protocol variability, patient-related factors, and limitations in current clinical evidence. Standardization of preparation parameters, personalization based on patient biology, and high-quality randomized controlled trials with long-term follow-up are essential to fully validate its regenerative potential and to establish evidence-based clinical guidelines for its predictable integration into contemporary oral regenerative therapy.

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