Advanced Platelet-Rich Fibrin: A New Concept for Cell-Based Tissue Engineering by Means of Inflammatory Cells

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Choukroun’s platelet-rich fibrin (PRF) is obtained from blood without adding anticoagulants. In this study, protocols for standard platelet-rich fibrin (S-PRF) (2700 rpm, 12 minutes) and advanced platelet-rich fibrin (A-PRF) (1500 rpm, 14 minutes) were compared to establish by histological cell detection and histomorphometrical measurement of cell distribution the effects of the centrifugal force (speed and time) on the distribution of cells relevant for wound healing and tissue regeneration. Immunohistochemistry for monocytes, T and B-lymphocytes, neutrophilic granulocytes, CD34-positive stem cells, and platelets was performed on clots produced from four different human donors. Platelets were detected throughout the clot in both groups, although in the A-PRF group, more platelets were found in the distal part, away from the buffy coat (BC). T- and B-lymphocytes, stem cells, and monocytes were detected in the surroundings of the BC in both groups. Decreasing the rpm while increasing the centrifugation time in the A-PRF group gave an enhanced presence of neutrophilic granulocytes in the distal part of the clot. In the S-PRF group, neutrophils were found mostly at the red blood cell (RBC)-BC interface. Neutrophilic granulocytes contribute to monocyte differentiation into macrophages. Accordingly, a higher presence of these cells might be able to influence the differentiation of host macrophages and macrophages within the clot after implantation. Thus, A-PRF might influence bone and soft tissue regeneration, especially through the presence of monocytes/macrophages and their growth factors. The relevance and feasibility of this tissue-engineering concept have to be proven through in vivo studies.

Key Words: PRF, neutrophils, inflammation, tissue engineering, platelets, macrophages

INTRODUCTION

A major objective of biomaterial research and tissue engineering is to promote a material-induced tissue reaction that leads to regeneration and an effective wound-healing process in the defective area. Thus, a biomaterial should serve as a temporary barrier to cover defects...
and promote tissue regeneration while being tissue compatible and, most importantly, clinically applicable. In the field of tissue regeneration, vascularization plays a crucial role as it ensures a continuous supply of nutrients to and the removal of waste products from the scaffold and the transplanted region.

Concepts such as the use of a biomaterial alone\(^1\,^2\) or preseeded with different primary mesenchymal\(^3\) or endothelial cells\(^4,^5\) are usual prerequisites for clinically applicable tissue engineering. However, concepts involving the precultivation of cells require time for cell isolation or cultivation as well as the possibility to aseptically handle more complex constructs in the operating room. These become major challenges if there is demand for a fast, robust, and “simple” approach by means of cell-based tissue engineering. Obviously, time is one of the most precious commodities in the clinic.

To ensure that methods for tissue engineering are widely applicable in the clinical field, it is necessary to modify them in a way that they are readily available and relatively easy to use within the daily clinical routine. Therefore, the steps between the preparation and application have to be minimized and optimized to make practical implementation realistic. Thus, it is the overall goal to develop concepts that are of natural origin, can be produced “close” to the patients, and would expedite the process of implantation while being financially realistic for the patient and the health system.

The requirements mentioned above led us to look for new strategies in which a new class of biomaterials is generated from autologous blood.\(^6^-^9\) Platelet-rich plasma (PRP) was the first generation scaffold derived from human blood samples, and this concept was widely studied and established with common denominators such as the addition of anticoagulants and bovine serum and double/-centrifugation.\(^10\) Comparative studies showed that PRP has a positive effect on the wound-healing process and tissue regeneration.\(^10\) However, the addition of anticoagulants and bovine serum limits the clinical application of PRP and calls for alternative, clinically feasible strategies.

With the aim of improving and streamlining these preparation methods—especially towards cell-seeded biomaterials generated from the patient’s own blood—a concept of platelet-rich fibrin (PRF) was developed.\(^11^-^16\) This fibrin scaffold, which does not possess any cytotoxic potential,\(^17\) is obtained from 9 mL of the patient’s own blood after 1 centrifugation step. The factors previously used in the preparation of blood-based scaffolds, such as anticoagulants or bovine serum, were excluded from this preparation procedure, which minimized the risk of trans-contamination. The main approach was to keep the methodology convenient and applicable for clinical use.\(^11^-^16\) The three-dimensional fibrin network is capable of mimicking the extracellular matrix in terms of its structure,\(^18,^19\) which creates the environment for cells to function optimally.

Choukroun’s PRF is derived from human blood and contains a variety of blood cells—including platelets, B- and T-lymphocytes, monocytes, stem cells, and neutrophilic granulocytes—as well as growth factors.\(^20,^21\) A major advantage of this method is its simplicity of preparation. The centrifugation process activates the coagulation process and as a result the clot is formed. This clot consists of a 3-dimensional fibrin network in which the platelets and other blood cells are entrapped. The release of growth factors from the PRF clot commences 5 to 10 minutes after clotting and continues for at least 60 to 300 minutes.\(^21,^22\) Several studies with Choukroun’s PRF have shown the tissue regenerative potential of this cell-loaded 3-dimensional scaffold. Interestingly, this scaffold is also a carrier for mesenchymal cells. Ling He et al were able to show that different cell types, such as rat osteoblasts, could differentiate and proliferate when cultured on the leukocyte-rich PRF (L-PRF). Differentiation and proliferation rates were investigated in terms of transforming-growth factor \(\beta\) (TGF-\(\beta\)), platelet-derived-growth factor AB (PDGF-AB), and alkaline phosphatase (ALP) activity at 5 time points. The study showed a slow but constant release of TGF-\(\beta\)1 and PDGF-AB as well as ALP activity.\(^23\)

Further experimental studies demonstrated that even dermal pre-keratinocytes, human gingival fibroblasts, pre-adipocytes, and maxillofacial osteoblasts underwent differentiation and proliferated with Choukroun’s PRF. Dental pulp cells were also able to grow and undergo further “differentiation” on the fibrin scaffold.\(^24\) In addition to the experimental approaches discussed above, clinical applicability of the material was tested. Mazor et al
showed that biopsies of the augmented/implant area taken 6 months after implantation revealed new bone formation, thus highlighting its possible osteo-inductive potential. Although the studies mentioned above underline the considerable potential of PRF in terms of tissue regeneration and clinical application, it was still not clear how cells are distributed in this type of scaffold depending on varying centrifugation time and speed (ie, cumulative centrifugal force). The aim of the study was to assess histologically and histomorphometrically the cell distribution pattern of fibrin clots by applying the standard PRF (S-PRF) protocol with 12-minute centrifugation time and 2700 rpm as well as the new advanced PRF (A-PRF) protocol with 14-minute centrifugation time and 1500 rpm.

**MATERIAL AND METHODS**

**Production of Choukroun’s PRF**

The PRF scaffolds were prepared according to a previously published protocol. Four healthy (ie, with no history of anticoagulant usage) volunteers in an age range between 18 to 60 years participated in this study. For each individual, 4 tubes of peripheral blood were collected and immediately placed in a preprogrammed centrifuge (PC-O2, PROCESS for PRF, Nice, France). Centrifugation was performed according to the following two protocols: (1) standard PRF, sterile glass coated plastic tube (9 mL; 2700 rpm for 12 minutes) and (2) advanced PRF, sterile plain glass-based vacuum tubes (A-PRF10 tube) (10 mL; 1500 rpm for 14 minutes).

**Histological preparation**

**Tissue Processing**

After centrifugation, the clots were carefully retrieved from the tubes. The red blood cell (RBC) fraction was removed in such a manner that the bottom of the fibrin-rich clot was not damaged. This technique is described in more detail in the Choukroun protocol. An impression about the shape of the clot is given in Figure 1. Fibrin-based clots with the buffy coat (BC) and part of the RBC were subsequently fixed with 4% paraformaldehyde solution for 24 hours. After that, they were cut and placed along the longitudinal axis into embedding cassettes.

**Histology and Histochemistry**

For further microscopic analysis, the samples were chemically processed in an alcohol series and xylene as previously described. Subsequently, paraffin embedding was performed, and 10 sections of 2 to 4 µm thickness, were cut with a rotary microtome (Leica RM2255, Wetzlar, Germany) and affixed on charged glass slides (SuperFrost Plus, Thermo Scientific, Waltham, Mass). Before staining, samples...
underwent a deparaffinization and rehydration process by sequential immersion in xylene followed by descending concentrations of ethanol. Several histochemical and immunohistochemical staining methods were then performed, as described as follows:

Three samples were histologically stained with standard protocols for hematoxylin and eosin (H&E) and Masson-Goldner’s trichome technique, as these special histochemical staining methods allowed discrimination of the components of the clots, that is, between cells and matrix proteins such as fibrin.\(^1\),\(^2\) The other six sections were used to identify various markers (Table) by means of immunohistochemical staining in an autostainer (DAKO, Hamburg, Germany).

### Immunohistochemistry

After establishing the optimal antibody concentration for each of the above-mentioned markers (DAKO), the slides were placed in a rack and incubated in Tris-EDTA pH 8.0 at 96°C for 20 minutes. Subsequently, the slides were rinsed with running tap water to cool. Then, the slides were washed with TBS and transferred to the autostainer. Before starting the autostainer, the required antibodies and solutions were loaded into the autostainer according to the manufacturer’s instructions. The DAKO EnVision detection system was used. After autostaining, the slides were counterstained with hemalun for 30 seconds and washed with running tap water. Finally, the stained slides were cover-slipped with Aquatex water-based, mounting medium (Merck, Germany).

### Histological evaluation

Histological examination was performed using a light microscope (Nikon Eclipse 80i, Tokyo, Japan). High-resolution photographs were taken with a connected Nikon DS-Fi1/Digital camera and a Nikon Digital sight unit DS-L2.

### Histomorphometry

For histomorphometrical analysis, the six immuno-histochemically stained slides of each clot were scanned by means of a Nikon Eclipse 80i microscope fitted with an automatic scanning table (Prior Scientific, Rockland, Maine), a Nikon DS-Fi1 digital camera and a computer with the Nikon NIS – Elements AR software, version 4.0. This combination allowed the capture of single high-resolution images and subsequent assembly of single images to one complete image (ie, a “total scan”).

The following histomorphometrical analyses of the comparative cell penetration within the clots were done also using the measurement function of the NIS – Elements software. Therefore, the total length of every clot was measured at first (in micrometer), and after that, the “penetration” lengths of the following cell types were also determined (in micrometer): stem cells, T- and B-lymphocytes, neutrophilic granulocytes, platelets, and monocytes. Finally, the distribution of the

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<th>Immunohistochemical markers and the specification of their use in the present study</th>
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<td><strong>Antibody</strong></td>
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different cell types was put into context within the respective clot length, thus allowing expression of the "penetration" lengths in percent (ie, "relative cell penetration") for the subsequent statistical comparisons.

Statistical evaluation

Statistical analysis was carried out by using the data of the two different experimental groups (ie, with \( n = 4 \) samples in each group). Penetration depths were then compared statistically. The obtained data yielded a mean ± S.E.M. with the aid of 1-way analysis of variance and a Bonferroni multiple comparisons post-hoc test via the GraphPad Prism version 6.0 (GraphPad Software, La Jolla, Calif). Thereby, inter-individual as well as intra-individual statistical significance were reported as significant (*) respectively at \( P < .05 \), and as highly significant (**) at \( P < .01 \) and (***) at \( P < .001 \). Finally, the quantitative data were presented graphically as the mean ± standard deviation (SD).

RESULTS

Histochemical studies (H&E, Mason-Goldner, and Giemsa)

S-PRF

In the longitudinal section of the S-PRF clot, produced according to the standard centrifugation protocol (2700 rpm, 12 minutes), a dense fibrin clot was seen with minimal interfibrous space. With the standard histochemical staining methods, cells were observed throughout the clot, albeit decreasing toward the more distal parts of the PRF clot (data not shown).

A-PRF

PRF clots formed with the A-PRF centrifugation protocol (1500 rpm, 14 minutes) showed a looser structure with more interfibrous space, and more cells could be counted in the fibrin-rich clot. Furthermore, the cells were more evenly distributed throughout the clot as compared to S-PRF, and some cells could be found even in the clot’s more distal parts. A representative image for cell distribution within A-PRF is given in Figure 2.

Descriptive immunohistochemical evaluation

S-PRF

The slides stained for the respective markers were evaluated in terms of specific cell type distribution (Figures 3 and 4). In general, the highest number of positively labeled cells was present in close proximity to the RBC or in the BC. In particular, cells with positive immunolabeling—including T-lymphocytes (CD3-positive cells), B-lymphocytes (CD20-positive cells), stem cells (CD34-positive cells), and monocytes (CD68-positive cells)—were found at the transition zone of the RBC fraction, BC, and proximal parts of the clot. The platelets (CD61-positive cells) were distributed throughout the clot. However, decreased numbers were observed from the proximal (near the BC) to the distal part of the S-PRF clot. Additionally, neutrophilic granulocytes (CD15-positive) showed a tendency to accumulate mainly in the RBC-BC clot interface.

A-PRF

Analogous to the S-PRF, slides were stained immunohistochemically for the markers of interest (Figures 3 and 4). Most of the CD3-, CD20-, CD34-, and CD68-positive cells stained in or near the BC (ie,
the very proximal part of the fibrin clot); however, the BC was more extensive in comparison to S-PRF. Additionally, the neutrophilic granulocytes (ie, CD15-positive cells) were distributed more widely toward the distal (ie, away from the BC) part of the fibrin-clot. Approximately two-thirds of the clot was seeded with neutrophilic granulocytes/CD15-positive cells, with only the last third (distal part of the fibrin clot) spared. As has been observed in the S-PRF clot.

**FIGURE 3.** Immunohistochemical reaction of the cells detected within the clots of the two experimental groups. Images S1–S6 show close-ups of the transformation zone between red blood cell (RBC), buffy coat (BC), and fibrin clot (FC) with different immunohistochemical staining for CD3-, CD15-, CD20-, CD34-, CD61-, and CD68-positive cells in the respective picture for platelet-rich fibrin (PRF) clots produced with the standard PRF protocol. As can be seen in the different images, the stained cells are mainly located within the border between the RBC count and the BC. Depending on the immunohistochemical marker, the cells are more or less prominent in the PRF clot. The red arrows mark the cells stained cells (positive) within the respective picture. The same applies for the Figure A1 through A6. These images are taken from PRF clots produced with the advanced PRF protocol.

**FIGURE 4.** Two total scans of the standard platelet-rich fibrin (PRF): left, hematoxylin and eosin (H&E) staining, total scan, 100 × magnification, and the advanced PRF; right, Masson-Goldner staining, total scan, 100 × magnification. Between both total scans, each immunohistochemical marker is portrayed by a colored bar (CD34–CD61). Within each of the total scans, the distribution of each cell type is depicted by separately colored bars. By looking at the total scans of both the standard and advanced PRF, it becomes apparent that CD61-positive cells (platelets) are distributed evenly throughout the clot, although the amount of platelets appears to decrease toward the peripheral parts of the clot in the S-PRF group. Additionally, the modification of the protocol resulted in an increased migration of CD15-positive cells into the A-PRF clot.
PRF group, platelets (ie, CD61-positive) were found throughout the entire clot. When compared to the S-PRF group, the amount of CD61-positive cells did not decrease to the same extent in the periphery.

**Quantitative histomorphometric analysis of cell penetration**

The histomorphometrical analysis of the total scans permitted an evaluation and comparison of the respective cell distribution in the PRF clots. The total length of each clot was measured and a mean of ± SEM was calculated. The distribution/allocation of each cell type was evaluated in the corresponding total scan of the immunohistochemical staining. The analyses revealed that platelets were the only ones found in each area of the clot up to 87 ± 13% in the S-PRF group and up to 84 ± 16% in the A-PRF group (Figure 5). Furthermore, the results showed that T-lymphocytes (S-PRF: 12 ± 5%, A-PRF: 17 ± 9%), B-lymphocytes (S-PRF: 14 ± 7%, A-PRF: 12 ± 9%), CD34-positive stem cells (S-PRF: 17 ± 6%, A-PRF: 21 ± 11%), and monocytes (S-PRF: 19 ± 9%, A-PRF: 22 ± 8%) were not found beyond a certain point of maximally 30% of the total clot length, as they are distributed in or near the BC generated by the centrifugation process (Figure 5). Statistical analysis revealed no statistically significant differences between both groups concerning the allocation of these cell types (Figure 5). Even though the experiments mentioned above show the promising capacities of this concept in terms of contribution to successful tissue regeneration, up until now, there have been no studies showing the extent to which the cells are distributed into the fibrin scaffold in relation to the two essential parameters centrifugation speed and centrifugation time.

In the present work, we have shown that T-lymphocytes, B-lymphocytes, stem cells, and monocytes are found in both groups within the first 25–30% proximal part of the clot. There are no statistically significant differences between both groups in terms of distribution of those cells.

**Discussion**

Complex tissue engineering concepts have to be evaluated in terms of their clinical applicability. Thus, the overall goal should be to establish a method that could ideally be completed within a short time span before or during intended regenerative surgical procedures. Over the last few years, Choukroun’s PRF has proven to be a method that comes close to the ideal concept of guided ("smart") tissue engineering. As previously shown, this form of PRF contains growth factors such as TGF-β1 and PDGF-AB^23^ and can contribute to tissue regeneration in terms of osteoblast, prekeratinocyte, and gingival fibroblast differentiation.\(^{18}\)

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The tissue regeneration or repair process requires harmonious reaction of various types of cells, including immune response cells (neutrophils, macrophages, lymphocytes), epithelial cells, fibroblasts, and stem cells, as well as other cells relevant for the tissue in question. The PRF scaffold concept seems to be an ideal source of components for the healing process. An autologous blood-derived scaffold can be a unique source of hematopoietic stem cells (HSCs), which are of major importance in regenerative medicine. In the past decade, many studies highlighted the vast differentiation potential of HSCs. A recent review by Ogawa et al. presented strong evidence for the pluripotent capacity of HSCs and summarized work that has been done in this field. Apart from their ability to replenish the majority of cell types in the body, stem cells also play a role as immune modulators. They can target B-lymphocytes and stimulate antibody secretion, inhibit or even lead to apoptosis of T-lymphocytes, and induce immune tolerance.

Other cells that can be observed in these advanced fibrin clots are B- and T-lymphocytes. Lymphocytes are responsible for specific and nonspecific intervention in tissue response for injury, although they are not prominent in the first phase of tissue repair. A study by Boyce et al revealed that CD8+ T-lymphocytes decreased wound healing, whereas B-lymphocytes were associated with an increased healing.

In contrast to the cells mentioned above, platelets are distributed more evenly throughout the entire clot. It appears that a decrease in centrifugation speed and an increase in centrifugation time results in higher platelet concentrations in the distal part of clot, although this observation was not definitively proven. Platelets, providing the name for these fibrin-rich scaffolds (ie, platelet-rich fibrin) are not only present in the coagulation pathway or primary wound closure but also have a vast regenerative potential by releasing a broad spectrum of cytokines, chemokines, growth factors, and other mediators. Platelets are able to release, amongst others, molecules such as von Willebrand factor, P-selectin, fibronectin, VEGF, platelet-derived endothelial growth factor (PDEGF), vitronectin, and fibrinogen. With these different growth factors, adhesion molecules, and other mediators, platelets have the ability to initiate and modulate host immune responsiveness through influencing neutrophils, monocytes, and endothelial cells, as well as lymphocytes. Upon stimulation, platelets actively participate in pathogen detection, capturing, and sequestration. They can even induce the death of infected cellular targets. Thanks to this wide range of activities, platelets are visible at each step of regeneration.

Monocytes are also essential for tissue healing. They migrate into the inflamed area after the influx of neutrophils, where they then become macrophages. Macrophages are multifunctional cells that represent distinct phenotypes. They have substantial roles in foreign body response, osteogenesis, and angiogenesis as they respond to inserted biomaterials. Through expression of VEGF, PDGF, FGF, TGFβ and –, and other biologically active molecules (eg, BMP-2), macrophages support cell proliferation and tissue restoration following injury. They are seen throughout all the processes of tissue repair from early inflammation through tissue-remodeling and scar formation.

One of the most important findings of the present study is that changing the centrifugation protocol in terms of centrifugation time and speed leads to a different distribution pattern for neutrophilic granulocytes. Neutrophilic granulocytes are most commonly considered to be early inflammatory cells due to their phagocytic capacity, degranulation, and neutrophilic extracellular traps. However, recent studies have shown that neutrophilic granulocytes have tissue regeneration properties as well. Neutrophils also facilitate trafficking of monocytes into the wound to phagocytose inflammatory remnants (necrotic and apoptotic cells). Moreover, they also participate in the process of wound debridement by secreting several proteases, including matrix metalloproteinase 9 (MMP9), an extracellular matrix digesting enzyme. Furthermore, neutrophilic granulocytes expressing MMP9 play a part in the process of revascularization of the tissue defect by being recruited, for example, by VEGF-A.

Neutrophilic granulocytes and monocytes/macrophages are in mutual communication, and their interplay contributes to further differentiation towards a pro- or anti-inflammatory state of the macrophages. Additionally, recent studies of Tan et al. revealed a potential contribution of neutrophils to inflammatory lymphogenesis by VEGF-A modulation and VEGF-D secretion in a
murine model. These cells modulate the innate as well as adaptive immune response in a direct and indirect manner by crosstalk with B- and T-lymphocytes. Thus, the distribution of neutrophilic granulocytes within the A-PRF clot might be the basis for a better functionality of the transplanted (but also resident) monocytes/macrophages and lymphocytes and their deployment to support tissue regeneration.

Aside from the neutrophilic granulocyte’s role in early inflammation and its potential regenerative properties, it has to be questioned why granulocytes are capable of migrating deeper into the matrix of the clot. One approach to addressing this question is the neutrophilic diameter. Thus, with an average diameter of 8.5–10 μm neutrophilic granulocytes are remarkably smaller than monocytes (diameter of 15–20 μm) and therefore could be more prone to penetrate deeper into the clot during the centrifugation process. If this, however, is true, it needs to be determined whether the weight of neutrophils correlates with their diameter. Basically, differential centrifugation is based on the differences in the sedimentation rate of the (biological) particles of difference size, shape, and density, as well as conditions of centrifugation. However, this present study failed to uncover why the neutrophilic granulocytes, in particular, “react” toward modifications in the centrifugation protocol. Therefore, further research has to be performed to validate these findings and give a scientific explanation for the different neutrophil behavior.

Among the ideas that are presently being established in the field of tissue regeneration—such as the use of co-cultures or monocultures, for example with osteoblasts or fibroblasts—the PRF concept could be an additional component suitable for clinical applications. This concept of generating a fibrin-based cell-seeded matrix solely by drawing blood and centrifugation for 12–14 minutes is truly revolutionary in terms of clinical practicability, as it can be handled and modified easily in a short period of time and provides the defect not only with a matrix permitting cell migration into the defected area but also providing the wound with crucial biological cues, potentially accelerating the wound-healing process: These include platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β), platelet factor 4 (PF4), IL-1, vascular endothelial growth factor (VEGF), epidermal growth factor, endothelial cell growth factor (ECGF), platelet-derived endothelial growth factor (PDGF), insulin-like growth factor, osteocalcin, osteonectin, fibrinogen, vitronectin, fibronectin, and thrombospondin. Furthermore, the fact that it is a matrix that is generated by the patient’s own (ie, autologous) blood, there is virtually no risk of a rejection reaction (foreign body response).

The future of clinically applicable tissue engineering concepts will most likely be spearheaded by cell-based tissue constructs that can be generated in a short time span and in close proximity to the patient. Nevertheless, it still has to be determined how these constructs, including Choukroun’s variation of PRF, will interact in vitro and in vivo after combination with cultured osteoblasts, fibroblasts, or a mixture with osteoconductive granules in vivo. It still remains to be shown in well-designed clinical studies that this fibrin-based matrix can facilitate guided bone-tissue engineering with simultaneous avoidance of obstacles such as infection, rejection, and cumbersome isolation methods. It is hoped that such clinical studies will also reveal to what extent the neutrophilic granulocytes contribute to the regenerative properties of the PRF.

**CONCLUSION**

The present data show that specific cell types are distributed differentially depending on the (cumulative) centrifugal force. This concept enables the optimal scaffold or composites to be tailored for specific clinical applications. These powerful composites can contribute to wound healing and tissue repair as well as tissue regeneration. Additionally, A-PRF appears to be an ideal provider of autologous cells (especially neutrophils and macrophages), thus enabling mutual stimulation, thereby creating a synergistic relationship in the interest of tissue regeneration.

**ABBREVIATIONS**

ALP: alkaline phosphatase  
ANOVA: analysis of variance  
A-PRF: advanced platelet-rich fibrin  
BC: buffy coat  
H&E: hematoxylin and eosin
HSC: hematopoietic stem cells  
L-PRF: leukocyte- and platelet-rich fibrin  
PDEGF: platelet-derived endothelial growth factor  
PDGF-AB: platelet-derived growth factor AB  
PRF: platelet-rich fibrin  
PRP: platelet-rich plasma  
RBC: red blood cell  
S-PRF: standard platelet-rich fibrin  
TGF-β: transforming growth factor  
VEGF: vascular endothelial growth factor

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