Optimized Platelet-Rich Fibrin With the Low-Speed Concept: Growth Factor Release, Biocompatibility, and Cellular Response

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Background: Over the past decade, use of leukocyte platelet-rich fibrin (L-PRF) has gained tremendous momentum in regenerative dentistry as a low-cost fibrin matrix used for tissue regeneration. This study characterizes how centrifugation speed (G-force) along with centrifugation time influence growth factor release from fibrin clots, as well as the cellular activity of gingival fibroblasts exposed to each PRF matrix.

Methods: Standard L-PRF served as a control (2,700 revolutions per minute [rpm]-12 minutes). Two test groups using low-speed (1,300 rpm-14 minutes, termed advanced PRF [A-PRF]) and low-speed + time (1,300 rpm-8 minutes; A-PRF+) were investigated. Each PRF matrix was tested for growth factor release up to 10 days (eight donor samples) as well as biocompatibility and cellular activity.

Results: The low-speed concept (A-PRF, A-PRF+) demonstrated a significant increase in growth factor release of platelet-derived growth factor (PDGF), transforming growth factor (TGF)-β1, epidermal growth factor, and insulin-like growth factor, with A-PRF+ being highest of all groups. Although all PRF formulations were extremely biocompatible due to their autogenous sources, both A-PRF and A-PRF+ demonstrated significantly higher levels of human fibroblast migration and proliferation compared with L-PRF. Furthermore, gingival fibroblasts cultured with A-PRF+ demonstrated significantly higher messenger RNA (mRNA) levels of PDGF, TGF-β, and collagen1 at either 3 or 7 days.

Conclusions: The findings from the present study demonstrate modifications to centrifugation speed and time with the low-speed concept favor an increase in growth factor release from PRF clots. This, in turn, may directly influence tissue regeneration by increasing fibroblast migration, proliferation, and collagen mRNA levels. Future animal and clinical studies are now necessary. *J Periodontol 2017;88:112-121.*

KEY WORDS

Blood; blood platelets; fibrin; fibroblasts; regeneration; wound healing.

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Over 15 years ago, platelet-rich fibrin (PRF) was introduced as an autogenous source of blood growth factors that could serve as a tool for tissue regeneration in modern medicine.[1] The concepts were derived from the fact that a first-generation platelet concentrate, platelet-rich plasma (PRP), was being heavily used in various fields of medicine despite bearing the negative aspect of containing anticoagulants, thereby preventing the full coagulation cascade important for tissue wound healing.[2-4] PRF (since renamed leukocyte PRF [L-PRF] due to its higher leukocyte content) does not contain anticoagulants and further provides a three-dimensional fibrin matrix that may be used as a scaffold for a variety of procedures including serving the function of a barrier membrane in guided bone regeneration and guided tissue regeneration procedures.[5-7]

Since its introduction in 2001,[1] PRF has been extensively used in dentistry for a variety of procedures, and its effectiveness has been demonstrated for extraction socket management,[8] gingival recessions,[9-11] intrabony defect regeneration,[12,13] and sinus elevation procedures.[7] Major advantages include having completely immune-compatible growth factors collected at relatively no costs without anticoagulants.[14-17] While initial and early experiments revealed PRP contained high concentrations of autologous growth factors (up to 6 to 8 times higher than normal blood concentrations), including platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and transforming growth factor (TGF)-β1,[18] PRF has since been shown to release even higher total growth factors over a more extended period of time.[19]

One primary proposed reason for a slower release of growth factors over time is the ability of the fibrin matrix to hold proteins within its fibrin network as well as cells capable of further release of growth factors into their surrounding microenvironment.[6,20-23] Leukocytes have been shown to be highly important immune cells capable of directing and recruiting various cell types during the wound healing process.[24-26] Since high centrifugation forces are known to shift cell populations to the bottom of collection tubes (whereas PRF is collected from the top one-third layer), it was recently hypothesized that by reducing centrifugation speed (G-force), an increase in leukocyte numbers may be achieved within the PRF matrix.[27] It was since shown that with decreased centrifugation G-force (now termed advanced PRF [A-PRF]), an increase in total leukocyte numbers within PRF matrix scaffolds was observed.[27] Furthermore, and in agreement with this hypothesis, it was shown that the release of several growth factors, including PDGF, TGF-β1, VEGF, epidermal growth factor (EGF), and insulin-like growth factor (IGF), were significantly higher in A-PRF compared with L-PRF and PRP.[19]

Since centrifugation force has been shown to have a direct impact on growth factor release from within PRF scaffolds,[19] the aim of the present study is to further investigate whether centrifugation time would similarly further improve growth factor release from within PRF scaffolds. In principle, less centrifugation time would reduce cell pull-down by centrifugation forces, which would theoretically increase the total number of cells left contained within the top layer (PRF matrix). Furthermore, since at present it remains completely unknown what influence these changes to centrifugation protocols will have on tissue regeneration, effects of each PRF matrix, including L-PRF, A-PRF, and A-PRF+, were investigated for the first time on human gingival fibroblast (HGF) cell biocompatibility and cell activity. Cells were therefore cultured with growth factors from each PRF matrix (L-PRF, A-PRF, and A-PRF+) and investigated for cell migration, proliferation, growth factor release, and collagen synthesis in vitro.

MATERIALS AND METHODS

The Institutional Review Board (IRB) of Nova Southeastern University (Fort Lauderdale, Florida) determined that this study did not require IRB review or approval.

Platelet Concentrations

Blood samples were collected with written informed consent of eight volunteer donors, aged 30 to 60 years (24 total samples). Blood was then processed for L-PRF, A-PRF, and A-PRF+ centrifugation. All blood samples were obtained from members of the authors' laboratory. Ten milliliters of whole blood without anticoagulant was centrifuged at 2,700 revolutions per minute (rpm) (708 × g) for 12 minutes for L-PRF; at 1,300 rpm (200 × g) for 14 minutes for A-PRF; and at 1,300 rpm (200 × g) for 8 minutes for A-PRF+, respectively, by a centrifuge machine.[#19,27] Size and volume of L-PRF, A-PRF, and A-PRF+ clots were produced in the top 4-mL layer of the centrifuge tubes (4 out of 10 mL). The PRF clot was removed and placed into a six-well dish with 5 mL of Dulbecco modified Eagle medium (DMEM) culture media** and processed as further described according to a previous study by the authors.[19]

Protein Quantification With Enzyme-Linked Immunosorbent Assay (ELISA)

To determine the amount of growth factors released from L-PRF, A-PRF, and A-PRF+ at 15 minutes, 60 minutes, 8 hours, 1 day, 3 days, and 10 days, samples were placed into a shaking incubator at

Duo Centrifuge, Process for PRF, Nice, France.
** Gibco, Thermo Fisher Scientific, Waltham, MA.

37°C to allow for growth factor release into the culture media. At each time point, the 5 mL of culture media was collected, frozen, and replaced with 5 mL of additional culture media. Protein quantification was carried out using ELISA. At desired time points, PDGF-AA (DY221, range = 15.60 to 1,000 pg/mL), PDGF-AB (DY222, range = 15.60 to 1,000 pg/mL), PDGF-BB (DY220, range = 31.20 to 2,000 pg/mL), TGF-β1 (DY240, range = 31.20 to 2,000 pg/mL), VEGF (DY293B, range = 31.20 to 2,000 pg/mL), EGF (DY236, range = 3.91 to 250 pg/mL), and IGF-1 (DY291, range = 31.20 to 2,000 pg/mL) were quantified using an ELISA kit[††] according to manufacturer protocol as previously described.[19] Absorbance was measured at 450 and 570 nm using a microplate reader,[‡‡] and the measurement at 570 nm was subtracted from the reading at 450 nm. All samples were measured in duplicate, and eight independent experiments were performed for each platelet concentrate.

Cell Culture

Platelet concentrates including L-PRF, A-PRF, and A-PRF+ were incubated for 3 days on a plate shaker at 37°C as previously described.[19] Thereafter, conditioned media was collected and used in future experiments as 20% of the total volume. All cell culture experiments were cultured with 20% conditioned media (CM) in standard DMEM cell culture media containing 15% fetal bovine serum (FBS). HGFs[§§] were cultured in a humidified atmosphere at 37°C in growth medium consisting of DMEM,[‖‖] 10% FBS,[¶¶] and 1% antibiotics[##] and used for experimental seeding from passages 4 to 6. All cells were detached from tissue culture plastic using 0.25% EDTA-trypsin[***] prior to reaching confluency. Cells were seeded with 20% CM from L-PRF, A-PRF, and A-PRF+ and contained within growth medium at a density of 10,000 cells for cell viability and proliferation experiments and 50,000 cells per well for real-time polymerase chain reaction (PCR) experiments in 24-well plates. Control samples were cells seeded onto tissue culture plastic alone that contained 20% CM left for 3 days on a plate shaker at 37°C without PRF clots (completely blank samples). For experiments lasting longer than 5 days, medium was replaced twice weekly.

Cell Viability

At 24 hours after cell seeding, cells were evaluated using a live-dead staining assay[†††] according to the manufacturer protocol. Fluorescent images were quantified with an inverted fluorescent microscope.[‡‡‡] Thereafter, cells were expressed as percentages of live versus dead cells after cell culture growth with L-PRF, A-PRF, and A-PRF+.

Cell Migration Assay

The migration assay of HGFs was performed using 24-well plates and polyethylene terephthalate cell culture inserts with a pore size of 8 μm.[§§§] Platelet-conditioned media were filled into the lower compartment of the wells. After being starved in DMEM containing 0.5% FBS for 12 hours, 10,000 cells were seeded in the upper compartment. After 24 hours, cells were fixed with 4% formaldehyde for 2 minutes. Thereafter cells were permeabilized by acetone for 15 minutes and stained with hematoxylin solution[‖‖‖] for 20 minutes. The upper side of the filter membranes was rinsed and gently wiped with a cotton swab to remove cell debris. Numbers of cells on the lower side of the filter were counted under a microscope.[¶¶¶]

Proliferation Assay

HGFs were quantified using an 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt colorimetric assay[###] at 1, 3, and 5 days for cell proliferation as previously described.[28] At desired time points, cells were washed with phosphate-buffered solution and quantified using a microplate reader.[****]

Real-Time PCR Analysis

Total RNA was harvested at 3 and 7 days post-stimulating for HGFs to investigate messenger RNA (mRNA) levels of *TGF-β*, *PDGF*, and collagen1a2 (*COL1a2*). Primer and probe sequences for genes were fabricated with primer sequences according to Table 1. RNA isolation was performed using an RNA isolation kit.[††††] Real-time PCR was performed using an appropriate mix[‡‡‡‡] and quantified on a PCR system.[§§§§] The ΔΔCt method was used to calculate gene expression levels normalized to the expression of *GAPDH*.

Statistical Analyses

All experiments were performed in triplicate with three independent experiments for each condition. Means and standard errors were calculated, and data were analyzed for statistical significance using one-way analysis for cell viability and migration assay, two-way analysis of variance for ELISA, proliferation assay, and

†† DuoSet, R&D Systems, Minneapolis, MN.
‡‡ DTX880, Beckman Coulter, Brea, CA.
§§ HGF-1, ATCC, Manassas, VA.
‖‖ Gibco, Thermo Fisher Scientific.
¶¶ Gibco, Thermo Fisher Scientific.
Gibco, Thermo Fisher Scientific.
*** Gibco, Thermo Fisher Scientific.
††† Enzo Life Sciences, Lausen, Switzerland.
‡‡‡ IX51, Olympus, Tokyo, Japan.
§§§ Falcon, Corning, Corning, NY.
‖‖‖ Sigma-Aldrich, St. Louis, MO.
¶¶¶ IX51, Olympus.
Promega, Madison, WI.
**** DTX880, Beckman Coulter.
†††† High Pure RNA Isolation Kit, Roche, Basel, Switzerland.
‡‡‡‡ Roche Master mix, Roche.
§§§§ StepOnePlus real-time PCR system, Applied Biosystems, Thermo Fisher Scientific.

Table 1.

List of Primer Sequences for Real-Time PCR

Gene	Primer Sequence
hTGF-β F	actactacgccaaggaggtcac
hTGF-β R	tgcttgaacttgtcatagatttcg
hPDGF F	cacacctcctcgctgtagtattta
hPDGF R	gttatcggtgtaaatgtcatccaa
hCOL1a2 F	cccagccaagaactggtatagg
hCOL1a2 R	ggctgccagcattgatagtttc
hGAPDH F	agccacatcgctcagacac
hGAPDH R	gcccaatacgaccaaatcc

real-time PCR analysis with Tukey test (P values <0.05 were considered significant) by relevant software.

RESULTS

Growth Factor Release From PRF, A-PRF, and A-PRF+

In a first set of experiments, release of growth factors including PDGF-AA, PDGF-AB, PDGF-BB, TGF-β1, VEGF, EGF, and IGF-1 were quantified by ELISA (Figs. 1 and 2). While the growth factor release of PDGF-AA demonstrated a significant increase for A-PRF+ at 3 days compared with L-PRF (Fig. 1A), no difference in total growth factor released was observed among the three treatment groups (Fig. 1B). Release of PDGF-AB demonstrated a significant increase for A-PRF+ at 8 hours compared with L-PRF and at 3 and 10 days compared with all other groups (Fig. 1C). L-PRF demonstrated significantly lower values than A-PRF and A-PRF+ at both 1 and 3 days (Fig. 1C). Total growth factor release was significantly higher for A-PRF+ compared with all modalities, whereas L-PRF was significantly lowest (Fig. 1D). Similarly, release of PDGF-BB demonstrated significantly highest values at almost all time points for A-PRF+, with L-PRF once again demonstrating significantly lower values compared with A-PRF and A-PRF+ (Figs. 1E and 1F).

Release of TGF-β1 demonstrated a similar trend whereby A-PRF+ demonstrated the significantly highest values at 1, 3, and 10 days (Fig. 2A), and the total release of growth factors after a 10-day period was almost three times significantly higher compared with L-PRF (Fig. 2B). Interestingly, A-PRF+ demonstrated a significantly higher release of VEGF at an early time point of 1 day (Fig. 2C), whereas little change was observed in the total growth factor release (Fig. 2D). Release of growth factors EGF and IGF-1 confirmed the low-speed concept favored release of both growth factors from A-PRF+ compared with A-PRF and L-PRF (Figs. 2E through 2H).

Biocompatibility of L-PRF, A-PRF, and A-PRF+ on HGFs

In a first cell culture experiment the influence of L-PRF, A-PRF, and A-PRF+ was investigated on cell viability of HGFs. It was found that all platelet formulations displayed excellent cell biocompatibility by demonstrating most notably high living cells (green cells, Fig. 3) with very few observable apoptotic cells (red cells). It may, therefore, be concluded from this experiment that each PRF formulation, including L-PRF, A-PRF, and A-PRF+, is fully biocompatible under the present in vitro cell culture model (Fig. 3).

Influence of PRF, A-PRF, and A-PRF+ on HGF Activity

After growth factor release experiments, each PRF formulation was investigated on HGF cell migration, proliferation, mRNA expression of growth factors, and collagen (Figs. 4 and 5). It was first observed that all PRF matrix scaffolds were able to significantly promote HGF migration at 24 hours, with L-PRF inducing a 200% increase, whereas A-PRF and A-PRF+ induced a 300% increase (Fig. 4A). No significant difference between A-PRF and A-PRF+ was observed, although both were significantly higher than control tissue culture plastic and L-PRF (Fig. 4A). A similar trend was also observed for cell proliferation, where each of L-PRF, A-PRF, and A-PRF+ significantly increased cell numbers at both 3 and 5 days compared with control tissue culture plastic, and A-PRF and A-PRF+ were significantly higher than all other groups at 5 days only (Fig. 4B).

Thereafter, mRNA levels of common regenerative cytokines PDGF and TGF-β were evaluated by real-time PCR (Fig. 5). When growth factor release of TGF-β from HGFs was investigated, although no difference was present at 3 days post-seeding, a significant increase was observed for all PRF groups at 7 days (Fig. 5A). A-PRF+ was found to demonstrate the significantly highest mRNA levels at 7 days compared with all other groups, including control tissue culture plastic, L-PRF, and A-PRF (Fig. 5A). A-PRF and A-PRF+ were found to provoke a slight significant increase in PDGF mRNA levels at 3 days compared with control tissue culture plastic (Fig. 5B). At 7 days, L-PRF demonstrated a significant increase compared with control tissue culture plastic, whereas both A-PRF and A-PRF+ once again provoked a significant increase in PDGF mRNA levels compared with all other groups (Fig. 5B). No significant difference was observed between A-PRF and A-PRF+ at either time point (Fig. 5B).

GraphPad Prism 6.0, GraphPad Software, La Jolla, CA.

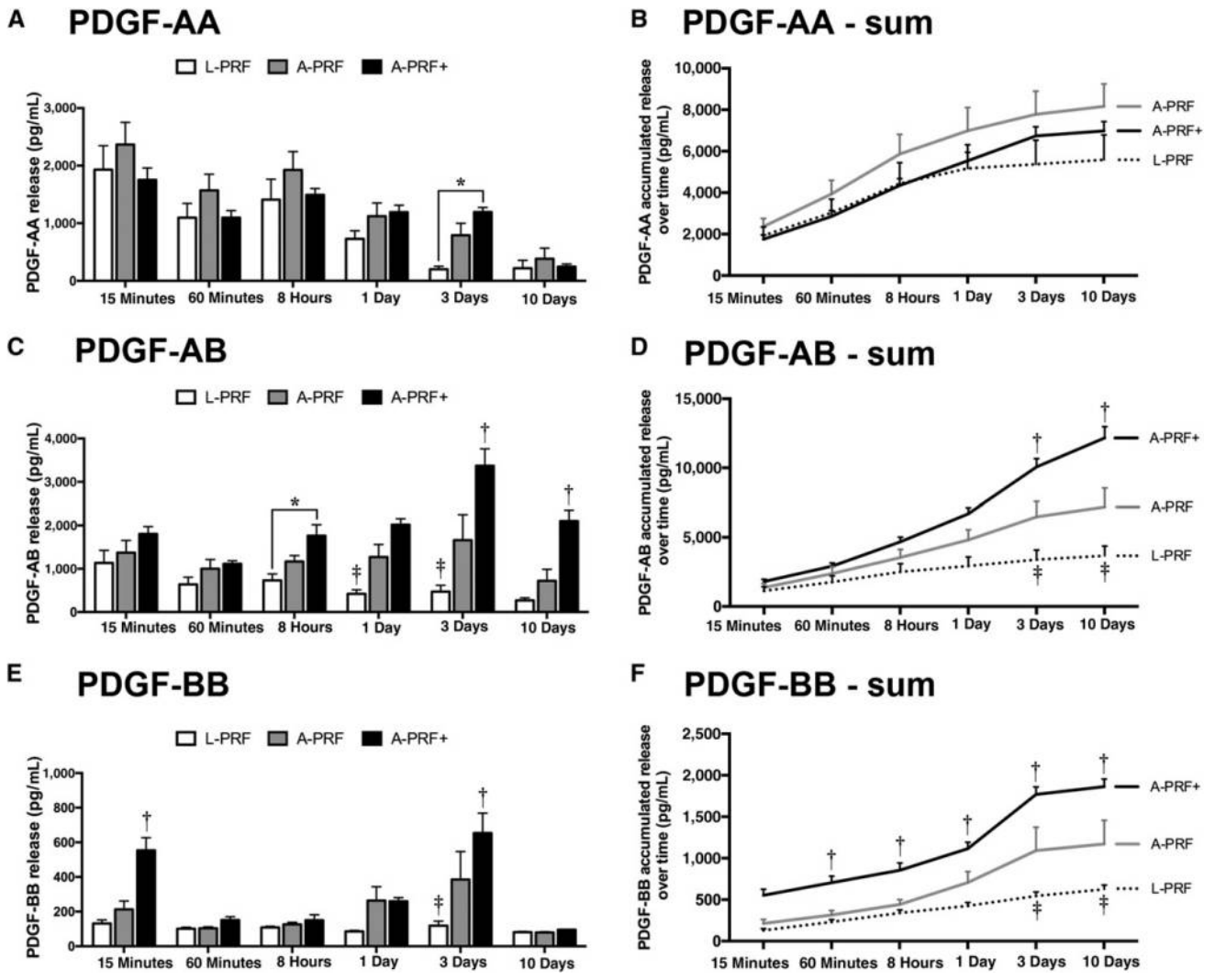

Figure 1.

*ELISA protein quantification at each time point of **A)** PDGF-AA, **C)** PDGF-AB, and **E)** PDGF-BB over a 10-day period. Total accumulated growth factor released over a 10-day period for **B)** PDGF-AA, **D)** PDGF-AB, and **F)** PDGF-BB. *P <0.05, significant difference among groups; †P <0.05, significantly higher than all other groups; ‡P <0.05, significantly lower than all groups.*

Lastly, collagen mRNA levels were quantified by real-time PCR (Fig. 5C). At 3 days, mRNA levels of collagen1 were significantly higher in A-PRF and A-PRF+ groups compared with control tissue culture plastic, with no significant differences observed for L-PRF (Fig. 5C). At 7 days post-seeding, L-PRF demonstrated significantly higher values compared with control tissue culture plastic, and A-PRF demonstrated statistically significantly higher values compared with control tissue culture plastic and L-PRF (Fig. 5C). A-PRF+ demonstrated the significantly highest mRNA values compared with all other groups (Fig. 5C).

DISCUSSION

The aim of the present study was to investigate influence of centrifugation speed (G-force) and time on PRF matrix scaffolds, their release of growth factors, as well as their effect on cellular biocompatibility and activity. As use of PRF has continuously and steadily increased in regenerative implant dentistry and periodontology, there remains great clinical benefit to optimizing centrifugation protocols for clinical practice. Therefore, the aim of the present study was to investigate if lower centrifugation speeds and time could be additionally used to improve growth factor release and cell bioactivity. An interesting finding from a previous study by Ghanaati et al.[27] was that in cells quantified histologically within the PRF matrix, the majority of leukocytes were found near the bottom of the fibrin clot in standard L-PRF. Based on this finding, it became clear that centrifugation speeds (G-forces) were evidently too high, pushing leukocytes to the

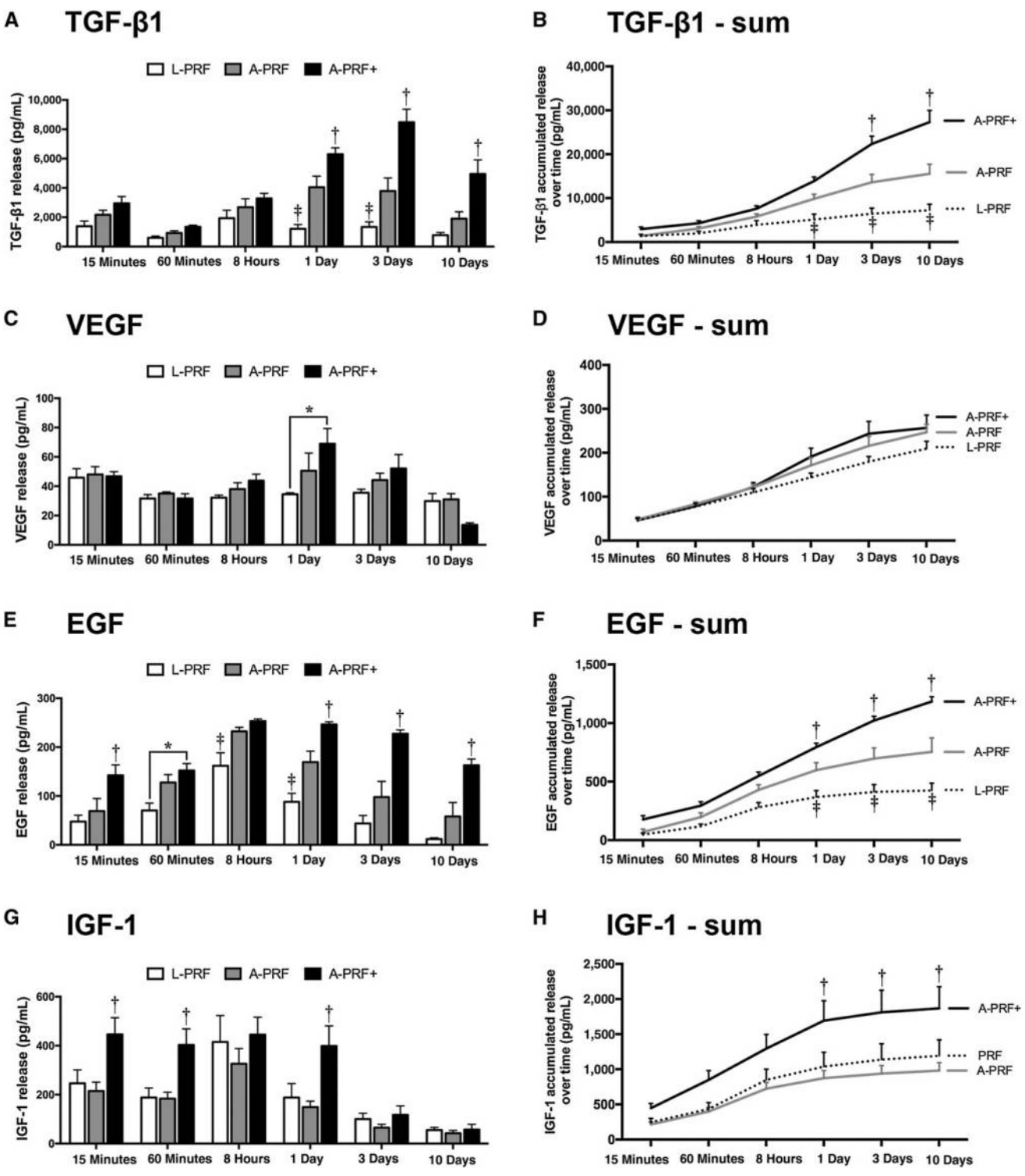

Figure 2.

*ELISA protein quantification at each time point of **A)** TGF-β1, **C)** VEGF, **E)** EGF, and **G)** IGF-1 over a 10-day period. Total accumulated growth factor released over a 10-day period for **B)** TGF-β1, **D)** VEGF, **F)** EGF, and **H)** IGF-1. *P <0.05, significant difference among groups; †P <0.05, significantly higher than all other groups; ‡P <0.05, significantly lower than all groups.*

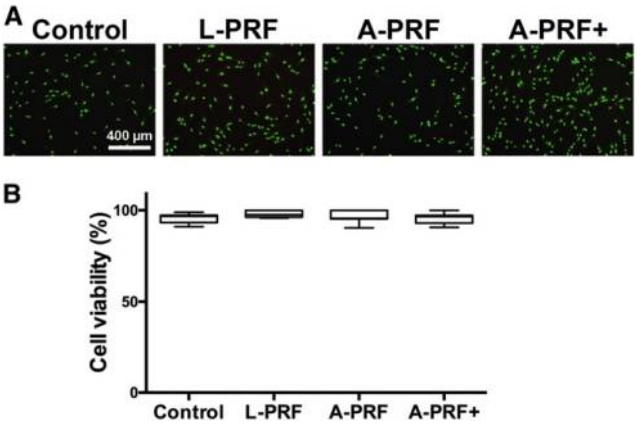

Figure 3.
Live/dead assay at 24 hours of HGFs treated with L-PRF, A-PRF, or A-PRF+. ***A)*** *Merged fluorescent images of live/dead staining with viable cells appearing in green and dead cells in red.* ***B)*** *Cell viability was quantified with percentage of numbers of living cells in each group. No significant changes in cell viability were observed for all platelet concentrates.*

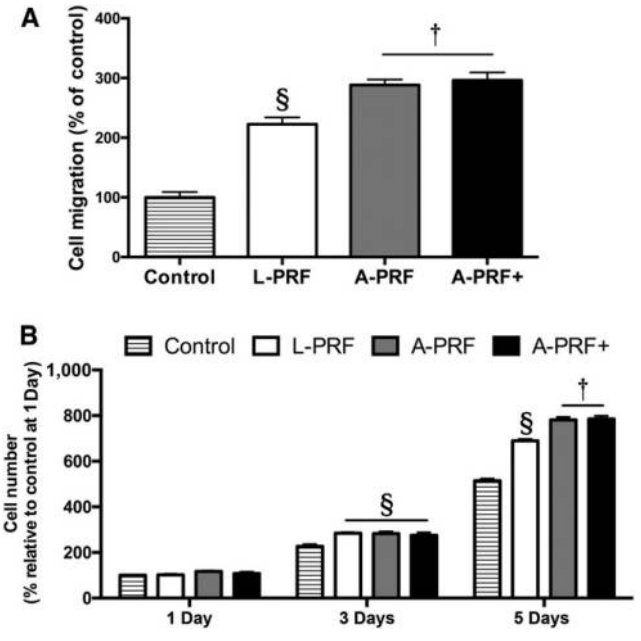

Figure 4.
Effects of L-PRF, A-PRF, and A-PRF+ on HGF ***A)*** *cell migration at 24 hours and* ***B)*** *cell proliferation at 1, 3, and 5 days.* †P <0.05, *significantly higher than all other groups;* §P <0.05, *significantly higher than control group.*

bottom of centrifugation tubes and away from the PRF matrix clot. To redistribute leukocyte cell numbers across the entire PRF matrix, lower centrifugation speeds were investigated.[27] It was confirmed that a higher cell number could be obtained by reducing G-force during centrifugation.[27] Ghanaati et al.[27] showed that although platelets were detected throughout the clot in both groups (L-PRF and A-PRF), more platelets were found in the distal part of A-PRF. Furthermore, by decreasing rpm while increasing centrifugation time in the A-PRF group, an enhanced presence of neutrophilic granulocytes in the distal part of the clot was observed.[27] Accordingly, it was reported that a higher presence of these cells might influence the differentiation of host macrophages and macrophages within the clot after implantation.[27] Thus, it was concluded that A-PRF might influence bone and soft tissue regeneration, especially through the presence of monocytes/macrophages and their growth factors.[27] In theory, the practical application of these new centrifugation protocols are derived by minimizing centrifugation speeds to limit the centrifugation pull-down of leukocyte cells to the lower compartment of centrifugation tubes. By reducing centrifugation G-force and time, a higher percentage of cells can, therefore, be collected within the top layer where PRF clots are located and used clinically. Histologic features and cell numbers of A-PRF+ compared with L-PRF and A-PRF remain to be investigated in a future study.

It must also be noted that the role of leukocytes in tissue wound healing and bone biology has been extensively discussed and is critically important to wound healing.[24-26] Interesting findings from basic science now point to the absolute necessity of macrophages during bone tissue remodeling[29] and have further shown that macrophages are responsible for a 23-fold increase in osteoblast differentiation.[30] Without these key immune cells, it has been shown that bone formation has very limited potential to generate new bone.[29] Furthermore, macrophages are key players in biomaterial integration and are the responsible cell type dictating material integration.[31] Therefore, it becomes evident that both an increase in leukocyte number as well as their even distribution across the PRF scaffold, as demonstrated with lower centrifugation speeds, is highly favorable during tissue wound healing and during biomaterial integration of collagen barrier membranes, various classes of bone grafting materials, and potentially dental implants.[31] Future research is therefore necessary.

Another important aspect of leukocyte biology that has not been discussed in this study, but again shows much clinical relevance, is the fact leukocytes are the responsible cell type acting to prevent infiltrating pathogens.[32,33] In light of this fact, it becomes of interest to note that PRF placed into extraction sockets has been shown to greatly decrease the rate of complications and infections.[8] Hoaglin and Lines[8] reported that filling third molar extraction sockets with PRF led to a 10-fold decrease in osteomyelitis infections compared with natural healing. This study, performed on 200 patients, used bilateral extractions (one side filled with PRF, the other left to naturally heal) and

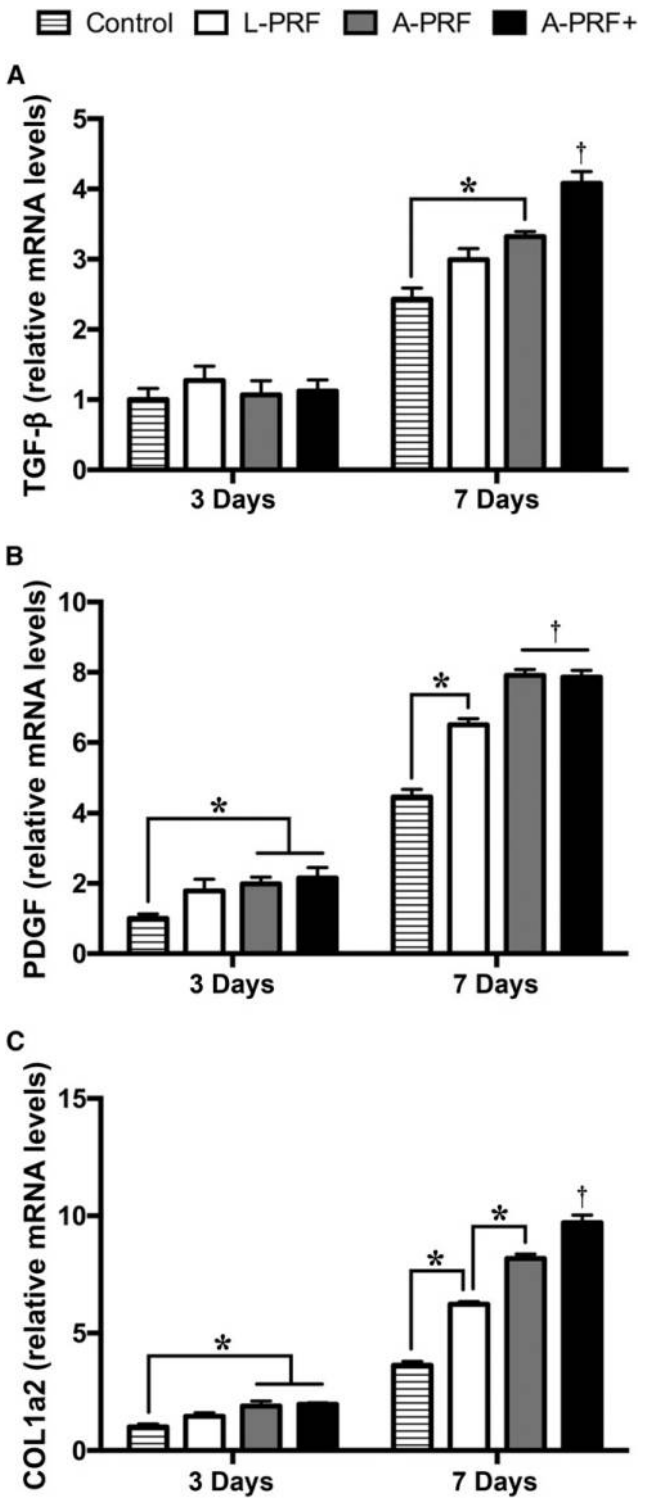

Figure 5.
*Real-time PCR of HGFs treated with L-PRF, A-PRF, or A-PRF+ at 3 and 7 days for mRNA levels of **A)** TGF-β, **B)** PDGF, and **C)** COL1a2. *P <0.05, significant difference among groups; †P <0.05, significantly higher than all other groups.*

provided good scientific evidence for the reduced rate of infection after healing with PRF.[8]

In the present study, growth factor release was first investigated from the various PRF matrix scaffolds produced by three different centrifugation protocols utilizing a slower-speed concept (Figs. 1 and 2). It was reported that A-PRF+ demonstrated significantly higher total growth factor release compared with A-PRF and L-PRF. It was, therefore, hypothesized that this finding is directly correlated with the fact that a higher number of leukocytes are found contained within the A-PRF+ scaffolds centrifuged using lower G-forces and centrifugation times. This finding alone is deemed highly clinically relevant, and these slight changes in centrifugation protocols were shown to have a direct and pronounced impact on growth factor release from within these A-PRF+ scaffolds. One aspect remaining to be investigated is how cytokine profiles of A-PRF and A-PRF+ compare with L-PRF. Since the most commonly found growth factors and cytokines in PRF are those investigated from previous work conducted over a decade ago from the original L-PRF formulation,[34] it remains of interest to determine if not only higher concentrations of growth factors are released from the various PRF formulations, but also if additional growth factors or cytokines may also be subsequently released. Future research using cytokine prolife assays comparing various PRF formulations would be necessary to further investigate these possible differences.

Another interesting area of research that is often left unstudied is the effect of higher-than-optimal doses of growth factors on tissue remodeling. For instance, Ohshima et al.[35,36] found certain growth factors, including TGF-β and VEGF, are not only capable of supporting tissue regeneration but may also participate in tissue degradation in periodontitis. While in general both of these growth factors are routinely associated with tissue regrowth (TGF-β) and angiogenesis (VEGF), it must not be excluded that they may also show negative effects. Future research investigating the optimal growth factor concentrations from PRF formulations remains to be conducted.

After analysis of growth factor release from PRF matrix scaffolds, this study sought to characterize the influence of L-PRF, A-PRF, and A-PRF+ on cell biocompatibility and cell activity (Figs. 3 through 5). It was first found that all PRF centrifugation protocols led to extremely high biocompatibility due to the autogenous source of these growth factors without use of anticoagulants (Fig. 3). Interestingly, it was found that A-PRF and A-PRF+ significantly promoted higher human gingival cell migration and proliferation compared with control tissue culture plastic and L-PRF (Fig. 4). Furthermore, analysis of mRNA levels of PDGF and TGF-β also demonstrated the ability for

PRF matrix scaffolds produced with the low-speed concept to significantly increase production of released growth factors from gingival fibroblasts. Therefore, not only are higher quantities of PDGF and TGF-β1 found in A-PRF+ scaffolds themselves (Figs. 1 and 2), but the cells then in contact with their matrix are also further stimulated to release more growth factors (Fig. 5), thus having a synergistic effect on the total growth factors produced locally.

Lastly, it was shown that A-PRF and A-PRF+ samples were able to locally increase collagen1 mRNA levels (Fig. 5C). Not surprisingly, collagen remains one of the key factors during tissue wound healing and remodeling.[37] Therefore, the increase in collagen Type 1 when cells were exposed to A-PRF and A-PRF+ further demonstrates the regenerative potential of the newer PRF formulations centrifuged at lower G-forces and lower centrifugation times.

CONCLUSIONS

The results from the present study demonstrate all formulations of PRF matrix scaffolds including PRF, A-PRF, and A-PRF+ were able to secrete the local release of various growth factors important for tissue regeneration. A-PRF+ demonstrated significantly higher release of growth factors compared with all other groups. Furthermore, A-PRF and A-PRF+ matrix scaffolds were shown to directly impact the ability of HGFs to migrate, proliferate, release additional growth factors, and increase mRNA levels of Type 1 collagen. Findings from the present study demonstrate modifications to centrifugation speed and time with the low-speed concept favoring an increase in growth factor concentrations directly impacting HGFs. Future animal and clinical studies are now needed to further confirm effects of these results in vivo.

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Drs. Fujioka-Kobayashi and Miron contributed equally to this work. Dr. Choukroun owns equity or stock options in Process for PRF (Nice, France). All other authors report no conflicts of interest related to this study.

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