Dayanjan Shanaka Wijesinghe, PhD

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*Correspondence: Dayanjan Shanaka Wijesinghe, PhD, Department of Surgery, Virginia Commonwealth University, 1101 East Marshall Street, P.O. Box 980614, Richmond, VA 23298-0614 (e-mail: wijesingheds@vcu.edu).

The Lipid Portion of Activated Platelet-Rich Plasma Significantly Contributes to Its Wound Healing Properties

Lauren Alexis Hoferlin,^{1,2} Quoc K. Huynh,² Jennifer A. Mietla,²
Scott A. Sell,^{1,3} Jason Tucker,¹ Charles Edward Chalfant,^{1,2,4,6}
and Dayanjan Shanaka Wijesinghe^{1,5,6,*}

¹Hunter Holmes McGuire Veterans Administration Medical Center, Richmond, Virginia.
Departments of ²Biochemistry and Molecular Biology and ⁵Surgery, Virginia Commonwealth University, Richmond, Virginia.

³Department of Biomedical Engineering, Parks College of Engineering and Technology, Saint Louis University, St Louis, Missouri.

⁴The Massey Cancer Center, Richmond, Virginia.

⁶Victoria Johnson Center for Critical Care Research, Virginia Commonwealth University, Richmond, Virginia.

Objective: Platelet-rich plasma (PRP) is a popular choice for the treatment of chronic wounds. Current dogma attributes these healing properties to the peptide growth factors of PRP. However, PRP is also rich in bioactive lipids whose contribution to healing has not been characterized and warrants investigation due to the protease-rich environment of chronic wounds.

Approach: The lipid fraction of PRP was tested with respect to proliferation and migration of primary adult human dermal fibroblasts (HDFa) ± exposure to chronic wound fluid (CWF). This fraction was also characterized via LC-MS/MS for bioactive lipids. A synthetic formulation of the bioactive lipid composition was developed and tested for the ability to overcome proliferative growth arrest induced by CWF.

Results: The data demonstrate the ability of the lipid fraction of PRP to significantly enhance the migration and proliferation of HDFa, and to overcome the proliferative growth arrest induced by CWF. Furthermore, the synthetic lipid formulation generated following characterization of the PRP lipidome demonstrated a similar ability to overcome proliferative arrest of HDFa in the presence of CWF.

Innovation: For the first time, we demonstrate the relevance of the lipid fraction of PRP toward the biology of wound healing. These studies open the possibility of altering the lipid profile of PRP via diet or exogenous pathway manipulation to obtain a better healing outcome.

Conclusion: The lipid fraction of PRP is under investigated and yet relevant component in wound healing. The current study demonstrates the relevance of this fraction in wound healing by PRP.

INTRODUCTION

PRESSURE ULCERS ARE DEFINED AS ischemia reperfusion injuries caused by unrelieved pressure, generally over a bony prominence, resulting in damage to underlying tissue.¹ These injuries induce tissue necrosis, which initiates an inflammatory cascade.

Neutrophils then infiltrate, which leads to an elevation of neutrophil-derived matrix metalloproteinases (MMP) at the wound bed, resulting in MMP-mediated tissue destruction.^{2,3} Loss of regulation in this sequence of events often results in an open chronic wound characterized by

the excessive and persistent presence of neutrophils.² Additionally, chronic pressure ulcers demonstrate very poor granulation, which can be attributed to fibroblast senescence. Indeed, fibroblasts isolated from these wound beds demonstrate a senescent phenotype and inefficiently respond to stimulatory signals.^{4–7} The combined effects of decreased matrix synthesis by senescent fibroblasts and increased matrix destruction by overabundant neutrophils cause the wound dimensions to increase. Thus, the principal requirements for the healing of pressure ulcers are both the reduction of neutrophils and an increase in migration and matrix deposition by fibroblasts.^{2,3,8}

Autologous platelet-rich plasma (PRP) is a treatment currently under evaluation to provide these principal requirements.^{9–13} PRP is defined as the portion of the plasma fraction of blood having a platelet concentration above baseline¹⁴ and PRP has been utilized in the treatment of many chronic skin and soft tissue ulcerations.¹⁵ Additionally, multiple publications demonstrate the use of PRP in wound healing related to various categories of surgery¹⁶ and burns.¹⁷ As platelets are a rich source of peptide growth factors¹⁸ [e.g., transforming growth factor beta (TGF- β) and platelet-derived growth factor (PDGF)], PRP would be enriched in these factors, and this would likely be advantageous for the wound healing process. To date, the beneficial effects of PRP have been solely attributed to these peptide growth factors. However, this hypothesis is paradoxical as published findings demonstrate that the chronic wound environment is highly proteolytic in nature and capable of rapidly degrading peptide growth factors.^{2,3} Therefore, the likelihood of the demonstrated healing potential of PRP being solely due to the peptide component is low. This suggests the presence of additional factors in PRP that are both resistant to proteolytic degradation and able to promote healing.

Many studies in the past three decades have established roles for lipid signaling molecules in multiple cellular biologies.^{19,20} Based on these studies and the above inferences, we explored the novel hypothesis that the lipid component of PRP plays a significant role in the wound healing process. Our data demonstrate the peptide-free lipid fraction of PRP to be pro-migratory and pro-mitogenic, and crucial to overcoming proliferative growth arrest of chronic wound fluid (CWF) with respect to adult human dermal fibroblasts (HDFa). Consequent lipidomic analysis demonstrated PRP to be enriched in many lipid signaling molecules with wound healing potential. A synthetic mixture of these lipids demonstrated the ability to overcome

the proliferative growth arrest of fibroblasts in the presence of CWF, confirming our hypothesis with respect to the healing potential of lipids in PRP.

CLINICAL PROBLEM ADDRESSED

While treatment of chronic wounds with PRP is fast becoming a staple in the available treatment repertoire, its true mechanism of action is only partially elucidated. As such, knowledge regarding mechanisms for improving the efficacy of PRP treatment is incomplete. In this regard, this study is an attempt to bridge this knowledge gap and also to identify new lipid-based treatment options to heal chronic wounds.

MATERIALS AND METHODS

Preparation of PRP, platelet poor plasma, and whole blood

Peripheral venous blood (30 cc) was obtained from six healthy donors through venipuncture. A portion of the blood was processed to produce PRP and platelet poor plasma (PPP) using SmartPreP[®] automated system. The remainder was unprocessed and termed whole blood (WB). The fractions were then pooled and an aliquot from each fraction (1 mL) was activated by treating with CaCl₂ (10 mM) and human thrombin (1 U). Following 30 min of incubation at 37°C, the mixture was centrifuged. The supernatant was stored in aliquots (200 μ L) under liquid nitrogen.

Extraction of lipids from PRP, PPP, and WB for treatment

An aliquot (200 μ L) was thawed on ice and diluted with molecular biology grade ethanol (1 mL) and sonicated on ice until homogenous. The resultant mixture was incubated overnight at –20°C to extract the lipids. The mixture was centrifuged at 4°C at 12,000 *g* for 20 min to remove insoluble material (e.g., precipitated proteins). Following centrifugation, the supernatant was carefully transferred to a new container and evaporated under a vacuum concentrator. Dried lipids were resuspended in molecular biology grade ethanol (40 μ L) for cell treatments. All lipid extractions were carried out in glass vials.

Harvesting of chronic and acute wound fluid

CWF was collected from discarded dressings that remained in contact with pressure ulcers overnight. The wound dressings were wetted with 2 mL of sterile phosphate-buffered saline and allowed to equilibrate for 2 h at room temperature followed by centrifugation at 6,000 *g* for 10 min. The clear supernatant was transferred into 1.5 mL microcentrifuge tubes and subjected to further

centrifugation at 15,000 *g* for 10 min to remove any residual cellular debris. The resultant solution was stored at 4°C and used within 48 h. Acute wound fluid was obtained from discarded Jackson–Pratt drain fluid. The collected fluid was aliquoted into 1 mL fractions and centrifuged at 15,000 *g* for 10 min to sediment all cells and cellular debris. This clarified acute wound fluid was stored at 4°C and used within 48 h.

Lipidomic analysis of PRP

Quantitative analysis of the lipids in the ethanolic extracts was carried out using UPLC-ESI-MS/MS as described with minor modifications.^{21,22} Briefly, to the acellular fraction of PRP, PPP, and WB (200 μ L), liquid chromatography mass spectrometry (LCMS) grade ethanol containing 0.05% BHT and 10 ng of each internal standard was added (1 mL). The internal standards used were as follows: (*d*₄) 6-keto PGF_{1 α} , (*d*₄) PGF_{2 α} , (*d*₄) PGE₂, (*d*₄) PGD₂, (*d*₄) LTB₄, (*d*₄) TXB₂, (*d*₄) LTC₄, (*d*₅) LTD₄, (*d*₅) LTE₄, (*d*₈) 5-hydroxyeicosatetraenoic acid (5-HETE), (*d*₈) 15-hydroxyeicosatetraenoic acid (15-HETE), (*d*₈) 14,15 epoxyeicosatrienoic acid, (*d*₈) arachidonic acid (AA), and (*d*₅) eicosapentaenoic acid. The samples were mixed using a bath sonicator followed by incubation overnight at –20°C for lipid extraction. Following incubation, the insoluble fraction was precipitated by centrifuging at 12,000 *g* for 20 min and the supernatant was transferred into a new glass tube. The lipid extracts were then dried under vacuum and reconstituted in of LCMS grade 50:50 EtOH:dH₂O (100 μ L) for eicosanoid quantitation via UPLC-ESI-MS/MS analysis. A 14 min reversed-phase LC method utilizing a Kinetex C18 column (100 $\times$ 2.1 mm, 1.7 μ m) and a Shimadzu Nexera UPLC was used to separate the eicosanoids at a flow rate of 500 μ L/min at 50°C. The column was first equilibrated with 100% Solvent A [acetonitrile:water:formic acid (20:80:0.02, v/v/v)] for 2 min and then 10 μ L of sample was injected. One hundred percent Solvent A was used for the first 2 min of elution. Solvent B [acetonitrile:isopropanol (20:80, v/v)] was increased in a linear gradient to 25% Solvent B to 3 min, to 30% by 6 min, to 55% by 6.1 min, to 70% by 10 min, and to 100% by 10.1 min. One hundred percent Solvent B was held until 13 min and then decreased to 0% by 13.1 min and held at 0% until 14 min. The eluting eicosanoids were analyzed using a hybrid triple quadrupole linear ion trap mass analyzer (ABSciex 5500 QTRAP[®]) via multiple-reaction monitoring (MRM) in negative-ion mode. Eicosanoids were monitored using species specific precursor $\rightarrow$ product MRM pairs. The mass spectrometer parameters used were as follows: curtain

gas: 30; CAD: High; ion spray voltage: –3,500 V; temperature: 500°C; Gas 1: 40; Gas 2: 60; declustering potential, collision energy, and cell exit potential were optimized per transition.

Treatment of primary HDFa

Primary human adult dermal fibroblasts were obtained from Invitrogen[®] (C-013-5C) and cultured as suggested by the manufacturer. For treatment, cells at passage 3 were plated at the required density and allowed to adhere and grow overnight under standard incubator conditions. On the day of treatment, the media was aspirated and replaced with media containing either the vehicle or the soluble lipid fractions of PPP, PRP, or WB. The treatment was repeated where necessary as indicated in the figure legends.

Scratch assay to investigate the migration of fibroblasts

Primary human adult dermal fibroblasts were obtained from Invitrogen (C-013-5C) and cultured as suggested by the manufacturer. For treatment, cells at passage 3 were seeded at a density of 5×10^5 cells per well for each well in a six-well plate. A reference line was drawn on the outside bottom surface of each well, and the cells were allowed to attach and spread overnight, creating an uninterrupted monolayer. Fifteen hours postseeding, a 200 μ L pipette tip was used to etch across the well perpendicular to the reference line creating an *in vitro* wound and the cells were treated with the test agents listed in the figure legends. Eighteen hours post-treatment the percentage of the exposed surface covered by cells was determined. The treatments were repeated as necessary as indicated in the figure legends.

Statistical analysis

Data are expressed as mean $\pm$ standard deviation (SD). Multiple comparisons were assessed using a one-way ANOVA with Tukey's *post hoc* method. A Levene's test was used to confirm equality of variances among the analyzed groups. Differences with a $p < 0.05$ were considered statistically significant. Statistical analysis was carried out using SPSS v22.0.0 statistical software (SPSS, Inc., Chicago, IL).

RESULTS

The lipid fraction of PRP enhances the migration of primary HDFa

Dermal fibroblasts conduct a primary role in the healing of cutaneous wounds. During the late inflammatory phase and the early proliferative phase of wound healing, fibroblasts migrate from the wound margins into the wound bed where they

proliferate and begin the synthesis of the new matrix.²³ Many lipids are known to influence the migration of fibroblasts,^{24–27} and as such, we investigated whether the lipids extracted from PRP will also demonstrate a similar induction of fibroblast migration. Monolayers of HDFa were subjected to a scratch assay and exposed to lipid extracts from pooled PRP, PPP, and WB obtained from six healthy donors. Eighteen hours post-wounding, the percent closure of the cleared area due to the migration of fibroblasts was assessed (Fig. 1). Compared to the fibroblasts treated with the vehicle control, the lipid extract from PRP induced a >2-fold increase in wound closure (Fig. 1). The fibroblasts exposed to the lipid extracts from WB or PPP failed to demonstrate a significant elevation in percent wound closure (Fig. 1). Taken together, the data indicate that the lipid component of PRP is a potent inducer of fibroblast migration. Furthermore, these data also demonstrate that the relevant lipid growth factors are present in a more potent combination in PRP compared to PPP and WB.

Lipids isolated from PRP are pro-mitogenic toward HDFa

In addition to migration into the wound bed, fibroblasts undergo a proliferative burst. This gen-

erates the required cellular cohort for the synthesis of the new matrix and other relevant signaling events. Suboptimal proliferation of fibroblasts results in decreased collagen synthesis, which often leads to the formation of decreased granulation tissue that delays wound healing. As many lipids are known to promote the proliferation of fibroblasts,^{24–27} we wanted to assess the ability of the lipid fraction of PRP in modulating the proliferation potential of fibroblasts. We treated primary human dermal fibroblasts with the lipid extracts from activated PRP, PPP, and WB (Fig. 2). A >2-fold elevation in the number of fibroblasts was observed after 24 h of treatment with the lipid fraction of PRP (Fig. 2). A much less pronounced, yet significant, increase was also observed for the lipid fraction of PPP (Fig. 2). However, the fibroblasts treated with the lipid component from WB demonstrated an insignificant change in proliferation compared to the vehicle control (Fig. 2). Taken together, these data indicate that the lipid fraction from activated PRP contains lipid mediators that are highly mitogenic toward primary human dermal fibroblasts. In contrast, the specific combination of lipid growth factors obtained from activated WB and PPP appear to be poorly conducive toward the proliferation of dermal fibroblasts.

Figure 1. Primary adult human dermal fibroblasts (HDFa) demonstrate enhanced migration in the presence of lipid extracts of platelet-rich plasma (PRP). HDFa cells (5×10^5) were seeded in each well of a six-well plate. A reference line was drawn on the outside bottom surface of each well, and the cells were allowed to attach and spread overnight, creating an uninterrupted monolayer. A 200 μ L pipette tip was used to etch across the well perpendicular to the reference line creating an *in vitro* wound. Cells were treated with a concentrated lipid extract (4 μ L) in ethanol added to the cell media (4 mL) so that the final concentration of lipid in the medium is equivalent to that from 20 μ L of PRP, platelet poor plasma (PPP), or whole blood (WB). Eighteen hours post-treatment the percentage of the exposed surface covered by cells was determined. Data are presented as the average percent coverage of wounds for six different observations $\pm$ standard deviation (SD). A one-way ANOVA followed by a Tukey's *post hoc* test was used to assess statistical significance between treatments. (** $p < 0.01$).

Figure 2. Primary HDFa demonstrate enhanced proliferation in the presence of lipid extracts of PRP. 1×10^5 HDFa cells were seeded onto each well of a six-well plate and allowed to adhere overnight under standard incubator conditions. Cells were then treated with a concentrated lipid extract (4 μ L) in ethanol added to the cell media (4 mL) so that the final concentration of lipid in the medium is equivalent to that from 20 μ L of PRP, PPP, or WB. Twenty-four hours post-treatment, the cells were removed from the plates via trypsinization and were counted using a Scepter hand held cell counter. Data presented are the average number of cells for six different observations $\pm$ SD. A one-way ANOVA followed by a Tukey's *post hoc* test was used to assess statistical significance between treatments. (** $p < 0.01$).

The lipid fraction of PRP “rescues” the proliferative growth arrest of fibroblasts induced by CWF

Multiple published observations illustrate that fibroblasts isolated from the wound bed of chronic wounds have significantly decreased proliferative ability. This is in stark contrast to those isolated from acute wounds, which undergo rapid proliferation in culture. This contrasting phenotype has been attributed to differences in the acute and CWFs. Since the lipid fraction of PRP is highly mitogenic toward the adult human fibroblasts (Fig. 2), we investigated whether the lipid fraction of PRP can overcome the growth arrest induced by CWF. In this regard, we cultured primary human dermal fibroblasts in the presence of acellular CWF plus the lipid fractions isolated from either PRP or PPP. In line with published reports, a significant decrease in the proliferation of fibroblasts was observed following 48 h of exposure to CWF compared with the vehicle-treated controls (Fig. 3). In contrast, acute wound fluid, as predicted, demonstrated a significant induction in fibroblast proliferation (Fig. 3). More importantly, the growth arrest of the fibroblasts was “rescued” by the lipid fraction of PRP, inducing levels of proliferation

Figure 3. Lipid extract of PRP overcomes the proliferative arrest in HDFa induced by chronic wound fluid (CWF). HDFa (1.5×10^5) were seeded onto each 60 mm plate and allowed to adhere overnight (15 h) under standard incubator conditions. At 15 h and again at 48 h postseeding, media was replaced with media containing the treatments. Cell free chronic and acute wound fluids were diluted 10-fold with media prior to treatment. Lipid extracts in ethanol equivalent to that obtained from 50 μ L of PRP or PPP were added to the relevant treatments. At 24, 48, and 72 h cells were removed from the plates via trypsinization and counted using a Scepter hand-held cell counter. Data presented are the average number of cells from six different observations $\pm$ SD. A one-way ANOVA followed by a Tukey's *post hoc* test was used to assess statistical significance between treatments. (** $p < 0.01$ compared to vehicle control).

similar to that of acute wound fluid (Fig. 3). While not as prominent, fibroblasts treated with the lipid fraction of PPP also managed to partially overcome the growth arrest induced by the CWF (Fig. 3). Taken together, these data demonstrate that the lipid fraction of PRP overcomes the proliferative growth arrest of fibroblasts in the presence of CWFs.

PRP is a rich source of bioactive lipids

Thus far, our data indicate that the lipid component of PRP potently induces the migration and proliferation of HDFa, which infers that bioactive lipids are present in a highly potent combination in activated PRP in contrast to activated PPP or WB. Knowledge of the specific lipids in activated PRP that contribute toward this healing phenotype would be of significant value in the generation of novel lipid-based treatment regimes to promote wound healing. To characterize the lipidome of PRP, we utilized a targeted and quantitative lipidomic analysis of PRP with respect to both eicosanoids and sphingolipids (i.e., lipids with proven bioactivities). This lipidomic analysis revealed a robust AA cascade as evidenced by significant elevations in AA, TXB₂, PGE₂, PGF_{2 α} , LTB₄, and LXA₄, in addition to 5-, 12-, and 15-HETE in PRP when compared with both PPP and WB (Fig. 4). Significant levels of S1P were also found in PRP. However, a significant difference in the levels of S1P was not observed between PRP and WB (Fig. 4H). These data indicate that PRP is a significant source of bioactive lipids with potential contributions to the wound healing mechanism.

Treatment with bioactive lipids identified in PRP from healthy donors allows fibroblasts to overcome CWF-mediated proliferative senescence

Our data with respect to primary HDFa have demonstrated that the lipid fraction of PRP can (a) enhance proliferation (Fig. 1), (b) enhance migration (Fig. 2), and (c) overcome proliferative arrest induced by CWF (Fig. 3). Furthermore, our lipidomic analysis of PRP revealed the presence of multiple bioactive lipids (Fig. 4). To identify the specific lipids responsible for overcoming this CWF-mediated proliferative arrest, we formulated a mixture consisting of AA, S1P, and 5-, 12- and, 15-HETE, chosen for their known bioactivities. In agreement with our previous studies, HDFa cells demonstrated proliferative arrest in the presence of CWF and enhanced proliferation in the presence of acute wound fluid. Lipid extract obtained from PRP again demonstrated the ability to overcome the CWF-mediated growth arrest. In accordance with our hypothesis, the synthetic lipid mixture

Figure 4. PRP contains significant quantities of bioactive lipids. Peripheral venous blood (30 mL) was obtained from six healthy donors and was either used as is for WB or processed to produce PRP and PPP as detailed in the Materials and Methods section. Each fraction (1 mL) was subjected to activation via treatment with CaCl_2 (10 mM) and human thrombin (1 U). Following 30 min of incubation at 37°C the mixture was centrifuged. Two hundred microliters of this supernatant was subjected to protein precipitation and lipid extraction as detailed in the Materials and Methods section. The resultant lipid fraction was analyzed using UPLC-ESI-MS/MS as described in the Materials and Methods section. Data presented are the average amount of lipids from six extractions $\pm$ SD. A one-way ANOVA followed by a Tukey's *post hoc* test was used to assess statistical significance between treatments. (** $p < 0.01$ compared to both PPP and WB, ## $p < 0.01$ compared to PPP, # $p < 0.05$ compared to PPP).

Figure 5. Synthetically formulated lipid blend identified from the lipid extract of PRP overcome the proliferative arrest in HDFa induced by CWF. HDFa (1.5×10^5) were seeded onto each 60 mm plate and allowed to adhere overnight (15 h) under standard incubator conditions. The following morning and 48 h later media was replaced with media containing the treatments. Cell free chronic and acute wound fluids were diluted 10-fold with media prior to treatment. Lipid extracts in ethanol equivalent to that obtained from 50 μ L of PRP or PPP were added to the relevant treatments. A synthetic lipid blend of arachidonic acid, S1P, 5-, 12-, and 15-HETE was formulated at the concentrations observed in PRP and similarly delivered in ethanol such that the amount added is equivalent to that from 50 μ L of PRP. At 24, 48, and 72 h cells were removed from the plates via trypsinization and counted using a Scepter hand-held cell counter. Data presented are the average number of cells from three different observations $\pm$ SD. A one-way ANOVA followed by a Tukey's *post hoc* test was used to assess statistical significance between treatments. (** $p < 0.01$ compared to vehicle control).

demonstrated a comparable ability to overcome the growth arrest induced by CWF (Fig. 5), and the cell numbers were well within the SD of that observed for the total lipid extract. The ability to overcome proliferative arrest is much reduced when treated with the individual lipids (data not shown). For the first time, this *in vitro* study demonstrates the potential for the use of synthetic lipid blends to induce proliferation in CWF-mediated growth arrested fibroblasts.

DISCUSSION

In this study, our laboratory investigated the contribution of the lipid component of PRP to its observed wound healing properties. Our data demonstrate that the isolated lipid fraction of PRP is capable of promoting the migration and proliferation of primary HDFa. Furthermore, this lipid fraction also exhibited remarkable potency in overcoming the CWF-induced proliferative growth arrest of dermal fibroblasts. Characterization of this lipid extract indicated the presence of many bioactive lipids that possess known functions related to the wound healing process. Thus, for the

first time, our laboratory has demonstrated the lipid fraction of PRP to be of biological relevance to both wound healing and relevant biological mechanisms.

We observed enrichment of TXB_2 (the stable metabolic product of TXA_2), $PGF_{2\alpha}$, LTB_4 , LXA_4 , and 5-, 12-, and 15-HETE in the lipid fraction of PRP in comparison to both PPP and WB. The enrichment of TXB_2 is of significance with respect to fibroblast migration as analogs of TXA_2 have been demonstrated to potentiate the chemotactic migration of lung fibroblasts.²⁸ Therefore, the decreased incidence of TXA_2 in WB would explain the decreased migration observed from this lipid extract compared to PRP. Also, in the context of cell migration, the high incidence of 12-HETE in PRP is relevant as previous studies have demonstrated that 12-HETE is a potent chemotactic agent for the migration of epidermal keratinocytes.²⁹ Future studies will focus on identifying which bioactive lipids are mediating fibroblast migration with an initial focus on 12-HETE and TXB_2 .

In this study we also found S1P to be elevated in PRP. This observation is in agreement with previous published findings³⁰ where it was shown that a thromboxane-dependent production of S1P is observed in platelets. The high incidence of S1P in PRP, and the induction of fibroblast migration in response to the lipid fraction of PRP, coincides well with previous observations where S1P was demonstrated to promote the chemotaxis of fibroblasts.³¹ On the other hand, the levels of S1P in WB and PRP are comparable, despite the contrasting biological effects on fibroblasts. Thus, a role for the S1P levels enriched in PRP in regards to fibroblast migration and proliferation is likely minimal, suggesting that the enriched eicosanoids are more biologically relevant.

Our studies also demonstrated that the lipid fraction of PRP "drives" the proliferation of fibroblasts. Indeed, the extract from PRP demonstrated a >2-fold increase in the number of proliferating human dermal fibroblasts. Among the lipids identified in PRP, 5-, 12-, and 15-HETE have been shown to be mitogenic toward fibroblasts.^{32,33} Elevated levels of $PGF_{2\alpha}$ in the lipid fraction could also be a contributing factor to the observed increase in HDFa proliferation, as the proliferative effects of increased $PGF_{2\alpha}$ synthesis has been demonstrated in both epithelial cells and NIH 3T3 fibroblasts.^{34,35} Several studies have shown that increased $PGF_{2\alpha}$ expression stimulates IL-6 synthesis and is proliferative in osteoblasts, thus highlighting this eicosanoid as an essential mediator in bone remodeling and fracture healing processes.³⁴ Another

report proposes that $\text{PGF}_{2\alpha}$ enhances osteoblast proliferation via regulation of basic fibroblast growth factor-2 (FGF-2) expression, which influences proliferation and differentiation by directly increasing the levels of cyclins D1, D2, D3, and E.³⁶ Moreover, FGF-2-induced proliferation has been demonstrated in cell types from epithelial, neuroectodermal, and mesenchymal origin.³⁶ Therefore, the increase in proliferation may be attributed to the high concentrations of these lipids in PRP.

A primary challenge in the treatment of chronic wounds is the persistent incidence of neutrophils in the wound bed. This maintains the wound in a permanent inflammatory state and inhibits the normal course of wound repair. In this context, our finding that LXA_4 is elevated in the lipid fraction of PRP is relevant toward explaining the pro-healing properties of PRP. Published studies demonstrate LXA_4 to decrease neutrophil infiltration into a wound microenvironment while at the same time enhancing the recruitment of nonphlogistic monocytes.¹⁸ The generation of LXA_4 likely occurs via a trans-cellular biosynthesis process where LTA_4 generated by neutrophils passively diffuses into the platelets, and is, in turn, acted upon by platelet 12 lipoxygenase³⁷ to produce LXA_4 . The process of PRP generation will bring the platelets and neutrophils into close proximity, allowing for this transcellular synthesis. Additionally, the presence of exogenous 15-HETE (present in abundance in PRP) has been demonstrated to enhance the synthesis of another class of lipoxins (LXB_4) by neutrophils.³⁸ The high levels of LXA_4 in PRP, and the additional synthesis of LXB_4 , in conjunction with neutrophils present in the wound bed may be responsible for PRP-induced transitioning of a chronic wound from its chronic inflammatory state into a granulating/proliferative state.

Our data also demonstrate high levels of LTB_4 in activated PRP, which is paradoxical to aiding the healing of chronic wounds. This bioactive lipid induces increased infiltration of neutrophils to the wound environment. As the presence of high levels of 15-HETE and LXA_4 would aid in suppressing the neutrophil chemotaxis in response to LTB_4 , the biological effect of LTB_4 may be minimal in most PRP treatments.³⁹ On the other hand, one may make the hypothesis that the ratio of LTB_4 to 15-HETE and LXA_4 may determine the effectiveness of PRP treatment in the clinic. If future studies validate this hypothesis, lipid analysis of PRP may allow for additional quality control in regards to this therapeutic regime. More importantly for the future of these studies is the actual development of a new therapeutic based on a synthetic li-

KEY FINDINGS

With respect to primary human dermal fibroblasts, the lipid portion of PRP was found to do the following:

- Promote proliferation.
- Promote migration.
- Overcome proliferative growth arrest in the presence of CWF.

pid cocktail rather than PRP use, which would remove the unwanted bioactive lipids and subsequently alleviate this issue.

In conclusion, we have demonstrated a direct relevance for the lipid component of PRP in biological mechanisms related to wound healing. As the lipidome is heavily influenced by variables such as disease state, medication, and diet, bioactive lipids may account for the high variability observed in the response of patients to autologous PRP treatment in the clinic. Therefore, the current study highlights the need of PRP preparation procedures to be adjusted (i.e., quality controlled) to maximize the output of the bioactive lipids for autologous PRP therapy. This study also implicates the possibility of the future production of a standardized, lipid-based therapy for the treatment of chronic wounds. At the very least, the presented study rationalizes additional lipidomic analyses of effective PRP regimes versus noneffective and relevant preclinical studies using the lipid component of PRP.

INNOVATION

For the first time, we have demonstrated the biological relevance of the lipid portion of PRP in promoting wound healing. This study leads to the possible alterations in the process of PRP generation with a focus on optimizing the presence of the lipid component to achieve greater potency towards healing.

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AUTHOR DISCLOSURE AND GHOSTWRITING

No competing financial interests exist. The content of this article was expressly written by the authors listed. No ghostwriters were used to write this article.

ABOUT THE AUTHORS

Lauren Alexis Hoeflerlin, PhD, received her degree in Biochemistry from the Medical University of South Carolina. She is currently an NIH F32 Postdoctoral Fellow in the Chalfant laboratory at VCU. **Charles Edward Chalfant, PhD**, received his degree from the University of South Florida and received his postdoctoral training at MUSC. Currently, Dr. Chalfant is a tenured Professor and the Vice Chair of the Department of Biochemistry and Molecular Biology at VCU, the Director of VCU Lipidomics/Metabolomic Core, and a Research Career Scientist at the Hunter Holmes McGuire VA Medical Center. His research focuses on C1P and its regulation of cPLA₂ α and on alternative splicing mechanisms in cancer. **Dayanjan Shanaka Wijesinghe, PhD**, received his degree in Biochemistry from the Virginia Commonwealth University (VCU) School of Medicine. Thereafter as an NIH F32 Postdoctoral Fellow, he began investigating the role of lipids in wound healing. In the meantime he successfully obtained funding for his wound healing research in the form of a CDA1 from the Department of Veterans Affairs at which point he transitioned to the VA as a Research Scientist. Currently, Dr. Wijesinghe is an Assistant Professor in the Department of Surgery at VCU where his primary focus of research continues to be the role of lipids in the regulation of wound healing. He is also the Assistant Director of the VCU Lipidomic/Metabolomic Core Facility. In

addition to his wound healing research, Dr. Wijesinghe leads a multidisciplinary research group examining the role of lipids in human critical illness and injury.

Quoc K. Huynh is a Graduate Student at Virginia Commonwealth University's Chemical Biology program under the tutelage of Dr. Charles Chalfant. He is currently involved in the development of a High throughput Screening Assay to investigate the interaction between the lipid molecule ceramide-1-phosphate and the Group IV PLA2 enzyme. In addition, he is also investigating the role of lipids in inflammatory disorders.

Jennifer Mietla, PhD, received her degree in Biochemistry and Molecular Biology from Virginia Commonwealth University School of Medicine. She is currently a postdoctoral fellow in the Chalfant laboratory at VCU.

Scott A. Sell, PhD, is currently an Assistant Professor in Biomedical Engineering at Parks College of Engineering, Aviation, and Technology. He received his PhD in Biomedical Engineering in 2009 working under the tutelage of Dr. Gary Bowlin. Following his graduation, Dr. Sell completed a 3 year Polytrauma Research fellowship at the Hunter Holmes McGuire VA Medical Center in Richmond, VA. Scott conducts research in the areas of tissue engineering and regenerative medicine, particularly focusing on the potential for electrospinning to create extracellular matrix analogue scaffolds for dermal and musculoskeletal repair. He has also done extensive research on the incorporation and controlled release of platelet-rich plasma from electrospun scaffolds.

Jason Tucker, MD, graduated from Marshall University School of Medicine, completed an orthopedic surgical internship at the University of Tennessee and a Physical Medicine and Rehabilitation residency at Virginia Commonwealth University. He is currently leading an Osteoarthritis Platelet Rich Plasma Mechanism of Action Study at the VAMC in Richmond, VA, including among other analyses, the effect PRP has on lipids in a human in vivo diseased joint environment; he serves in a consultative role in other related research projects. He has published both journal articles and textbook chapters in the field of Orthopedic Regenerative Medicine.

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Abbreviations and Acronyms

AA	= arachidonic acid
CaCl ₂	= calcium chloride
CWF	= chronic wound fluid
FGF	= fibroblast growth factor
HdFa	= adult human dermal fibroblasts
HETE	= hydroxyeicosatetraenoic acid
LCMS	= liquid chromatography mass spectrometry
LTB ₄	= leukotriene B ₄
LXA ₄	= lipoxin A ₄
MMP	= matrix metalloproteinases
MRM	= multiple-reaction monitoring
PDGF	= platelet-derived growth factor
PGE ₂	= prostaglandin E ₂
PGF ₂ α	= prostaglandin F ₂ alpha
PPP	= platelet poor plasma
PRP	= platelet-rich plasma
S1P	= sphingosine-1-phosphate
SD	= standard deviation
TGF-β	= transforming growth factor beta
TXA ₂	= thromboxane A ₂
TXB ₂	= thromboxane B ₂
UPLC-ESI-MS/MS	= ultra performance liquid chromatography electrospray ionization tandem mass spectrometry
WB	= whole blood