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Autologous platelet-rich plasma for healing chronic venous leg ulcers: Clinical efficacy and potential mechanisms

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The overall quality of evidence of autologous platelet-rich plasma (PRP) for treating chronic wounds remains low. While further well-designed clinical studies are clearly required to convincingly demonstrate the efficacy of autologous PRP in improved healing of venous leg ulcers (VLUs) and other chronic wounds, there is also an increasing need to better define the underlying mechanisms of action and whether positive outcomes can be predicted based on the analysis of PRP. This brief review will discuss the current understanding of autologous PRP in VLUs and whether molecular evaluation of PRP at the time of collection could potentially be informative to clinical outcomes. Benefits of the autologous PRP treatment strategy include that PRP is easily accessible and is relatively inexpensive and safe. Better understanding of the mechanisms involved could improve treatment, enable supplementation, and/or lead to gains in product development. Analysis of PRP could also add value to future clinical trials on efficacy and potentially personalised treatment regimens.

KEYWORDS

platelet glycoproteins, platelet physiology, wound healing

1 | INTRODUCTION

Venous leg ulcers (VLUs) are the most common cause of lower limb ulceration in community settings in developed countries, with an incidence of 1.5 to 3.0 in 1000 in people aged 65 years and younger. Prevalence increases with age to reach 20 in 1000 in people aged older than 80 years.[1] In Australia, VLUs are the most common clinical chronic wound problem seen in community practice.[2] Sustained healing is a major challenge that has a considerable impact on individual health and quality of life. In 2012, there were approximately 47 299 cases of VLUs reported in Australian hospitals and almost 2000 in residential care settings.[3] Currently, standard treatment for leg ulcers is compression of the lower leg by bandaging to reduce hydrostatic pressure in the leg,[4] but even with best practice compression therapy, time to healing is often prolonged. New treatment options to improve time to healing are urgently needed.

Platelets play critical roles in wound healing, from haemostasis to initiation of wound repair, as well as release of cytokines and growth factors and the ongoing maintenance of vascular integrity.[5–7] A gel prepared from a patient's own blood is increasingly being used to facilitate improved wound healing in people with chronic wounds.[8,9] Despite promising results in a few studies, there is still a lack of high-quality research to prove its effectiveness.[10,11] In this review, we will discuss current evidence on the benefits and potential risks of using autologous platelet-rich plasma (PRP) gel (APG) as an adjunct to compression therapy in healing VLUs and consider some of the key molecular components and other factors in the blood that are potentially involved in promoting wound healing. Identifying

measurable blood properties may help to predict efficacy and tailor treatments.

2 | EPIDEMIOLOGY AND DISEASE BURDEN

Age-related VLU is the most common cause of lower limb ulceration in the western world, responsible for almost 75% of all leg ulcers. The burden and cost of VLUs are expected to rise dramatically because of an ageing population and increasing prevalence of diabetes and obesity. Ulcerative wounds are typically painful, malodorous, and weeping and are associated with a poor quality of life[12] and develop as a result of chronic venous defects because of dysfunctional valves or blockage of the veins causing long-term high pressure.[13] Factors that commonly contribute to poor healing include local causes such as wound infection, tissue hypoxia, trauma, and necrotic tissue, as well as systemic diseases such as diabetes.[14] Such factors could also contribute to the relative efficacy of autologous PRP. Medical treatment aims to improve blood flow to the area, and compression therapy is the current best practice treatment; however, healing is unpredictable, and ulcers frequently recur.[4] Almost a third of these patients experience more than 10 episodes of ulceration, and recurrence rates have been estimated to be between 50% and 80%.[15,16] Treatment of VLUs may also be compromised by delayed diagnosis, overuse of antibiotics, and insufficient or inadequate use of compression therapy.[2] Few other adjuvant treatments have been effective.[17,18]

In 2016, an updated analysis of randomised control trials for evidence supporting the use of autologous PRP for treating chronic wounds reported a total of 10, with 3 involving VLUs and 3 foot ulcers in individuals with diabetes.[11] While conclusions suggested that PRP might improve the healing of foot ulcers in diabetic cases, it is unclear whether PRP is beneficial for other chronic wounds, and improved clinical trials are needed.[11] One small, non-blinded study of 13 participants with VLUs that included both venous aetiology and diabetic foot ulcers did report that 50% of participants healed with the application of the gel-like APG, and those healed were more likely to stay healed for longer.[19] Along with additional larger trials, an improved understanding of the mechanisms by which platelets promote wound healing in vivo and as autologous PRP gels ex vivo, and the ability to predict therapeutic success or failure, is also now required. Furthermore, recommendations for the improvement of definitions, preparation, clinical trial design, and utility in different clinical scenarios of PRP have recently been published.[20] These recommendations will help unify the field and identify the most appropriate combinations of platelet bioactive factors to achieve maximal regenerative activity.

Key messages

- quality of evidence of autologous platelet-rich plasma (PRP) for treating chronic wounds remains low
- there is a need to better define the underlying mechanisms of action and whether positive outcomes can be predicted based on analysis of PRP
- a better understanding of the mechanisms involved could improve treatment

3 | AUTOLOGOUS PRP GEL

Autologous PRP is one's own blood plasma enriched with platelets.[21] Platelets play a pivotal role in primary haemostasis, rapidly adhering, becoming activated, forming aggregates, and promoting coagulation at sites of injury (within seconds to minutes) to seal a blood vessel and limit blood loss. Platelets also play a critical role in wound healing. During normal wound repair, activated platelets within the fibrin matrix release the contents of their storage granules, including growth factors important for wound healing, such as platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), epidermal growth factor (EGF), and vascular endothelial growth factor (VEGF).[7,22] In skin vasculature, during severe inflammation, platelets play a key role in endothelial repair and integrity, involving interactions between platelet receptors glycoprotein (GP)VI and C-type lectin-like receptor 2 (CLEC-2) with extravascular collagen and podoplanin on macrophages, respectively.[5] In contrast to the scenario in acute healing, chronic VLUs are arrested at the inflammatory stage. Non-resolution of inflammation and the presence of bacteria could result in wound exudates containing disordered cytokine/growth factor network and high levels of proteolytic enzymes that destroy tissue proteins, growth factors, and extracellular matrix.

APG is produced essentially from the addition of thrombin and divalent calcium ions to PRP, causing activation of platelets and clotting factors, liberation of growth factors, and the formation of a viscous fibrin-containing gel. APG has been used for >20 years to reduce blood loss in surgery and, more recently, has been used to promote wound and bone healing.[23,24] Approaches that trial the combination of APG and a biodegradable gelatin hydrogel in a wound dressing, aiming to provide sustained release and improve the biological half-life of growth factors from PRP, are ongoing.[25] The effects of APG have also been tested in a variety of animal models.[26] The main mechanism of action of APG is considered to be the release of growth factors to trigger a tissue regeneration process.[27] In this regard, an interesting recent study combined proteomic analysis of PRP with effects on skin ulcer wound healing ($n = 17$) and migration of various cell types (endothelial cells and precursors,

mesenchymal cells, fibroblasts) by analysis of cell markers and using cultured mesenchymal cells or fibroblasts and migration assays in vitro.[28] Some of the growth factors, as well as angiogenic and cell migration factors, that were highly expressed in PRP used for APG included PDGF-AA and -AA/BB; matrix metalloproteinase-9 (MMP-9); angiopoietin-1 and -2; VEGF; endostatin; angiogenin; endothelin-1; and insulin-like growth factor-binding protein (IGFBP)-1, −2, and − 3. Another study has quantified profiles of cytokines, chemokines, and growth factors in PRP from 16 healthy donors determining levels of more than 20 different factors, including high concentrations of the pro-inflammatory cytokine RANTES and gender differences in levels of PDGF-BB.[29]

The specific contribution of individual factors or a combination of factors or profiles to the clinical efficacy of APG has not yet been demonstrated in clinical trials. Such factors from PRP have also been investigated as media for growth and expansion of human bone marrow mesenchymal stromal cells, with PDGF-BB and bFGF being critical.[28,30] Some of the molecular pathways in target cells such as keratinocytes, which can result in cell cycle arrest and cell senescence in non-healing ulcers, have also been investigated.[31] There is also evidence that profiles of growth factors can vary depending on the agonist or process used to manufacture APG. For example, pulsed electric field activation of PRP at bipolar low versus monopolar high field strength results in differential platelet-derived microparticle production and activation of platelet surface pro-coagulant markers.[32,33] It is apparent that molecular contents of platelet releasates vary depending on the method used to induce platelet activation and degranulation (summarised in Table 1), indicating the type of variation that could occur depending on how APG is produced. It will be important to gain further insights that could also apply to the analysis of other measurable components of PRP in the future.

4 | FUTURE DIRECTIONS

Many properties of autologous PRP, including concentrations of relevant bioactive factors and platelets, and the function and reactivity/activation of markers that could potentially reflect the overall capacity of platelets to promote healing in vivo or when converted to APG are not well characterised.

One potentially useful platelet-specific marker is GPVI, a member of the immunoglobulin-like receptor family and primary platelet receptor for collagen and fibrin, and its metalloproteolytically shed ectodomain fragment, soluble GPVI (sGPVI) (reviewed in References [37–39]). Plasma sGPVI levels are independent of age and gender. Interestingly, sGPVI in plasma is elevated above healthy levels in vascular coagulopathy or microangiopathy, including disseminated intravascular coagulation (DIC), and in inflammatory arthritis.[40–43] Low to normal levels of sGPVI in plasma of PRP used for APG in VLUs, for example, could indicate suboptimal platelet reactivity; alternately, very high levels of sGPVI could indicate a loss of capacity to promulgate wound healing: further investigation of sGPVI and other markers in autologous PRP could usefully be analysed and correlated with other measurable factors (Table 1) as well as clinical outcomes in larger scientific-clinical studies.

Profiles of growth factors can vary depending on the agonist or process used to manufacture APG (above), and sGPVI levels might also provide further information on how activation influences wound healing in vivo or as APG. In inflammatory arthritis, the collagen receptor GPVI on platelets has been identified as critical for the generation of microparticles (extracellular microvesicles) released from activated platelets.[43] Other studies have also shown that different forms of microparticles with varying pathophysiological consequences may be released in an agonist-dependent manner.[42,44] Therefore, detailed examination of sGPVI and platelet-derived microparticles in PRP used for APG could be uniquely informative regarding wound-healing potency (Table 1). Platelet membrane microparticles can also transport translatable and/or small interfering RNA that can be transferred to inflammatory and/or other vascular cells that promote wound healing.[44–47] Determining if these factors also play a functional role in APG and/or predict its efficacy is important for future research in this field.

Another pro-inflammatory/pro-coagulant marker in human plasma is cell-free nucleosomal DNA, also referred to as neutrophil extracellular traps (NETs), that are extruded by neutrophils in response to infectious agents or other stimuli (reviewed in References [48–50]). NETs are composed of filamentous DNA providing a net-like structure and contain

TABLE 1 Contents of platelet releasates vary with the method used to induce platelet activation and degranulation

Released treatment	sGPVI	Growth factors, cytokines	Microparticles	Anticoagulant factors	References
No treatment	No	+	—	ND	32,34
Thrombin ± $CaCl_2$	No	++	++	++	28,32,35
Thrombin + collagen/convulxin	Yes	++	++	++++	34,36
Non-physiological agonists					
Phorbol esters, ionomycin	Yes	++	++	++	32
Electrolysis	ND	+++	+++	+++	

ND, not determined; Convulxin is a GPVI agonist purified from *Crotalus durissus terrificus* venom.

nuclear proteins such as chromatin and histone (H1, H2A, H2B, H3, H4) and secretable neutrophil serine proteases, neutrophil elastase and cathepsin G, and von Willebrand factor (VWF) through an interaction with histones. Interactions between NETs/VWF and platelets via the primary platelet VWF receptor GPIbα of the GPIb-IX-V complex leads to platelet activation and release of pro-inflammatory factors and growth factors, thereby recruiting additional neutrophils and amplifying the process. As discussed for plasma sGPVI (above), nucleosomal DNA levels in plasma could potentially reflect both the extent of the inflammatory response in vivo and predict the wound-healing capacity of PRP used for APG. In this respect, quantitation of the scope and variability of potential protein and cellular markers, secreted cytokines and growth factors, coagulation, and other regulatory factors mentioned above may aid standardisation and/or personalisation of treatments using APG and PRP.

There are also opportunities for future research to integrate information about individual diversity, including genomic diversity, to personalise treatment regimens that incorporate precision medicine principles. Genome-wide analyses of the factors discussed above could greatly complement the quantitative profiling of these components. Analysis of genotypes and/or transcriptomes related to these different components in PRP or other samples may provide a more complete (and likely complex) picture of how critical these known factors are alone and in combination, as well as having the potential to identify new factors that could be investigated in platelet/endothelial function and wound healing.

Finally, platelet hyper-reactivity in diabetes - where VLUs are more prevalent and the use of autologous PRP more indicated - is associated with an increased platelet intracellular oxidative state coinciding with elevated levels of reactive oxygen species (ROS) known to be generated downstream of GPIbα/GPVI engagement.[51–54] Whether such measurable parameters contribute mechanistically or predict poor wound healing in diabetic individuals, as well as the efficacy of autologous PRP in VLUs, has yet to be determined. In addition, although not considered in detail here, non-autologous allogeneic healthy donor PRP or serum, with associated quality control and product development to define levels of growth factors within optimal ranges, could also be relevant to the use of PRP in ulcers and provide a future alternative therapy to using autologous PRP.[55]

In conclusion, determining the relationship between APG efficacy and composition, ultimately identifying measurable blood properties, is highly likely to help predict the efficacy and outcome of such treatments. Analysis of multiple types of parameters in PRP used for APG could be combined in future clinical studies to improve clinical outcomes via molecularly informed and potentially personalised treatment regimens.

CONFLICT OF INTEREST

The authors have no conflicts of interest to report. There are no relevant funding sources to declare.

AUTHOR CONTRIBUTIONS

C.W., E.E.G., J.F.A., M.S., and R.K.A. wrote the paper and approved the final version. All persons designated as authors qualify for authorship. Each author has participated sufficiently in the work to take public responsibility for the content.

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