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Platelets as Regulators of Wound Healing

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ABSTRACT

Wound healing occurs through a well-orchestrated series of events encompassing hemostasis, inflammation, cellular proliferation and tissue remodeling. Platelets are specialized vascular secretory cells that rapidly accumulate at sites of injury, quickly becoming the most prevalent cell type. They undergo expansive activation within damaged tissue, initiated by the exposure of extracellular matrix proteins and by the local generation of thrombin. Platelets contain numerous granule types which hold more than 300 biological active components. The content of these granules are released upon platelet activation. A wealth of studies now demonstrates that the contents of these granules are able to regulate most stages of wound healing. In the past two decades an increased understanding of the physiological roles of platelets in wound healing has led to the idea of using platelet products as therapeutic tools to enhance skin repair. This article highlights the mechanisms by which platelets enhance wound closure and discusses the clinical effect of platelet therapy on wound healing.

Keywords: Platelets, Wound healing, Inflammation, Dermal fibroblasts, Keratinocytes, Clinical trials

INTRODUTION

Wound healing occurs through the interconnected stages of haemostasis, inflammation, migration/proliferation and remodelling as recently reviewed [1]. After the initial cessation of bleeding by haemostasis, the inflammatory phase begins. Neutrophils and monocytes enter the damaged tissue to remove pathogens and cellular debris. In addition to debridement, the inflammatory phase provides the initial burst of signaling mediators required to start the proliferative phase. The simultaneous recruitment of endothelial cells and fibroblasts is responsible for angiogenesis and the formation of granulation tissue, supported by the provisional extracellular matrix [ECM] secreted by fibroblasts.

Granulation tissue formation initiates basal keratinocytes migration and proliferation within the damaged tissue, allowing re-epithelisation to occur. Finally the remodelling phase occurs as the immature neo-matrix, laid down in preceding steps, is degraded in favour of the production of organised type 1 collagen fibres essential for tensile strength. During remodelling, fibroblasts differentiate into myo-fibroblasts to allow wound contraction, then as the ECM and normal tissue stratification is restored, apoptosis removes the excess differentiated cells, leading to wound resolution. Through the release of a wide range of cytokines, growth factors and pro-inflammatory mediators, platelets are able to impact on many of these responses.

Under physiological conditions platelets circulate in the blood in an inactive state, largely due to repression by nitric oxide and prostaglandin produced from vascular endothelial cells [2]. When the vascular integrity is disrupted, platelets rapidly come into contact with the newly exposed ECM. Platelet adhesion receptors for von-Willebrand factor and collagen support the initial tethering and subsequent firm adhesion of platelets respectively. Following this initial interaction, platelets undergo a well characterised series of events including integrin affinity up regulation, cytoskeletal re-organisation, and ultimately aggregation, resulting in the formation of a haemostatic plug. Platelet activation is strongly reliant upon the secretion of pro-thrombogenic mediators stored in platelet dense granules. In addition to dense granules, platelets contain a large number of α granules, which store over 300 bioactive compounds [3]. The contents of these include growth factors, adhesion molecules, clotting mediators, chemokines and inflammatory molecules. These released factors enable platelets to initiate and regulate angiogenesis, stimulate inflammation and induce target cellular growth. These responses place platelets in a central position to impact on wound healing, which is the focus of this review.

Platelets and inflammation

As the first responders to cutaneous injury, platelets become the most prevalent cell type in damaged tissue. Platelets circulate at around 300,000 cell/μl blood, accounting for a larger volume than all leukocytes combined [4]. Platelet α granules contain both adhesive receptors required to interact with leukocytes and a range of chemotactic and activatory cytokines. These signaling molecules are largely synthesised in pre-cursor megakaryocytes before being packaged in mature platelets, although some signaling mediators such as serotonin are taken up and stored by platelets from the plasma [5,6]. Additionally it is now clear that platelets can synthesise signaling proteins from stored mRNAs in response to physiological stimuli [7].

P-selectin

Perhaps the best studied of α granule content is P-selectin, an integral membrane glycoprotein rapidly redistributed to the

platelet plasma membrane following activation. P-selectin expression allows robust platelet binding to circulating monocytes, neutrophils and leukocytes through cognate P-selectin glycoprotein ligand [PSGL-1] [8]. Leukocyte PSGL-1 attachment to P-selectin on adherent platelets at sites of injury is critical for leukocyte rolling and subsequent firm attachment to the damaged tissue [9,10]. Additionally the trans-endothelial migration of neutrophils is at least in part dependent on platelet expressed P-selectin and its ligand PSGL-1 [11]. Platelet released serotonin greatly aids in P-selectin mediated leukocyte recruitment. Mice lacking platelet serotonin have decreased leukocyte rolling and attachment to the endothelium and a reduction in the recruitment of neutrophils to cutaneous injuries [12]. Furthermore PSGL-1 knockout mice display reduced early wound healing, decreased inflammatory cell infiltration and reduced growth factor expression within damaged tissue [13].

CD40L

The tumour necrosis factor [TNF] family member glycoprotein CD40 is strongly pro-inflammatory, regulating a range of processes in immune responses [14]. Activated platelets are the major source of circulating soluble CD40 ligand [CD40L] and express CD40L on their surface, which can induce endothelial expression of leukocyte adhesion molecules including E-selectin, vascular cell adhesion molecule [VCAM]-1 and intercellular adhesion molecule [ICAM]-1 [15]. Heightened levels of soluble CD40L are found in a range of inflammatory disorders including psoriasis, which is characterised by excessive and rapid growth of epidermal keratinocytes and a complex immune infiltrate [16]. CD40L signaling may contribute to the severity of psoriasis once an autoimmune response is initiated through altering the cytokine profile and adhesion molecule expression of keratinocytes [16-18]. Platelets circulate in a semi-activated state in patients with psoriasis [19], which has been suggested to help drive cutaneous inflammation [20]. It remains to be determined however if the enhanced platelet CD40L expression in psoriasis directly contributes to heightened inflammation around lesions.

Inflammatory cytokines

The inflammatory cytokines platelet factor 4 [PF4], regulated on activation of normal T cell expressed and secreted [RANTES], thymus and activation-regulated chemokine [TARC], interleukin

[IL]-1β and transforming growth factor [TGF]-β are plentiful in platelet α granules [21]. Their levels and time of release can help regulate wound healing or further enhance inflammatory conditions such as atopic dermatitis [AD]. TARC supports fibroblast and lymphocyte motility [22], supporting wound healing [22]. However elevated levels of TARC are found in the platelets of patients with AD, and may contribute to AD pathogenesis by aiding in the recruitment of Th2 T cells to lesions, thereby driving hyper-inflammation [23].

PF4 functions at much higher concentrations than other chemokines [24], but is found in abundance within platelets. PF4 largely works as a co-stimulator to induce neutrophil adhesion and secretion [25], aiding in the initiation of the inflammatory phase of wound healing. PF4 works in close association with RANTES to modulate immune cell recruitment [26]. Platelets deposit RANTES on inflamed endothelial cells in a P-selectin dependent manner, allowing for site specific monocyte arrest [27,28]. Platelet derived RANTES additionally can modulate the differentiation of T cells into a Th1/Th17/T regulatory phenotype [29], which are major producers of IL-17A, IL-17F, IL-21, and IL-22. These interleukins together provide a key defence against bacterial infection, but may also play a role in autoimmune disorders such as psoriasis [30].

In addition to storing IL-1β, platelets can synthesise new IL-1β from constitutive mRNA once activated [31]. Thus as the first cellular responders to tissue injury, platelets are likely to provide the initial IL-1β signal leading to inflammation. IL-1β plays an essential role in early response to infection [32] and increases adhesion molecule expression on leukocytes for endothelial cells, allowing increased adhesion and diapedesis [33]. IL-1 receptor-deficient mice exhibit impaired wound healing, attenuated collagen deposition and delayed neovascularization, highlighting its critical role [34]. If inappropriately regulated however IL-1β promotes the formation of the strongly inflammatory M1 macrophage sub-type, which is associated with delayed wound healing [35].

TGF-β1 has an important role in controlling the influx of immune cells to damaged tissue and is a principal regulator of macrophage M2 differentiation [36]. M2 macrophages produce anti-inflammatory cytokines, including IL-10, and aid in phagocytosis of apoptotic cells, together enabling the resolution of inflammation and tissue repair [37,38]. TGF-β1 additionally exerts anti-inflammatory effects [39,40] through the inhibition of T cell maturation and down regulation of macrophage inducible nitric oxide synthase expression [iNOS] [41]. iNOS produces large amounts of nitric oxide and subsequent ROS production, which can both contribute to neutrophil recruitment and direct tissue damage [42]. TGF-β also aids in mediating fibroblast migration, collagen deposition and granulation tissue formation, as well as differentiation of fibroblasts into contractile myofibroblasts, an essential step for wound closure [33,43]. A summary of the inflammatory effects of platelets can be found in Figure 1.
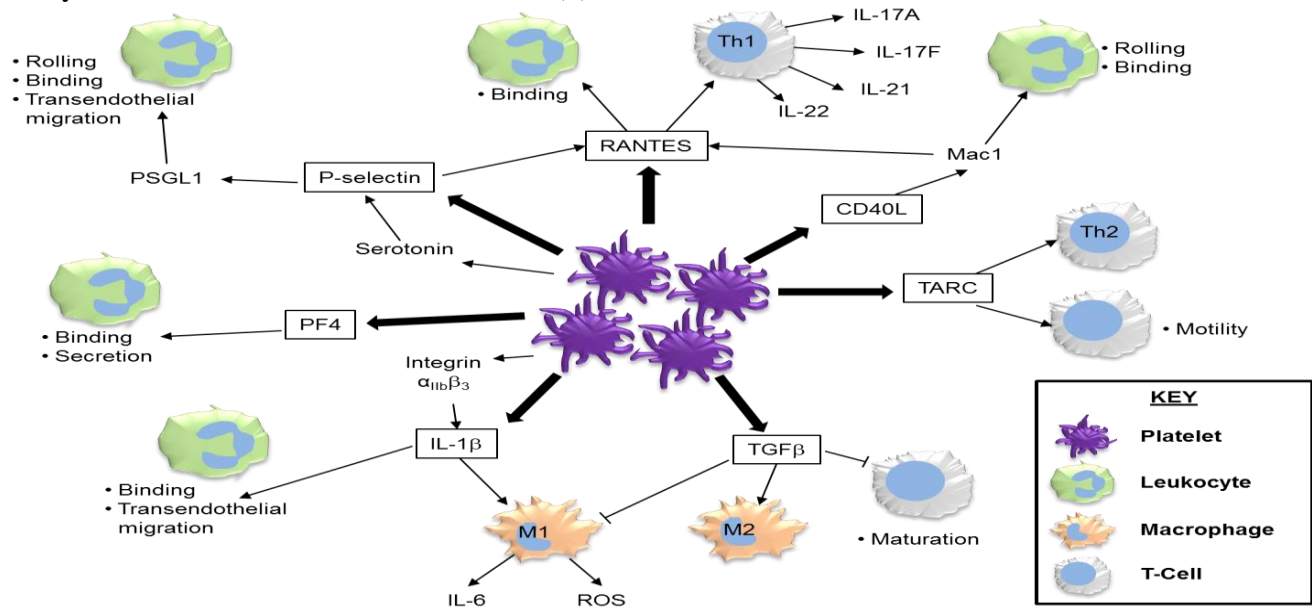
Figure 1: Platelets and inflammation. Activated platelets can trigger an array of both pro- and anti-inflammatory pathways, modulating the behavior of many white blood cells. Please see main text for details. Abbreviations: IL, interleukin; PF4, platelet factor 4; PSGL1, P-selectin glycoprotein ligand-1; RANTES, Regulated on activation normal T cell expressed and secreted; ROS, reactive oxygen species; TARC, thymus and activation-regulated chemokine; TGF, transforming growth factor.

Chronic wounds are characterized by a prolonged, robust inflammatory phase with elevated levels of macrophages, inflammatory mediators and tissue proteases. This dysregulation typically halts the start of the proliferative phase of wound healing. Patients with chronic venous, pressure and diabetic wounds have reduced levels of TGF-β1 [44-46] and an inability to exit the inflammatory phase.

Platelets promote fibroblast wound healing responses

In addition to their well-documented inflammatory role, growing evidence suggests that platelets help drive the proliferative phase of wound healing. Neurobeachin-like 2 gene knockout mice produce platelets deficient in α-granules, enabling them to be used to identify the role platelet degranulation plays in tissue regeneration. These mice display impaired healing and granulation tissue formation after excisional skin wound injury, with a decrease in myo-fibroblasts and collagen production [47,48]. The ability of platelets to impact on wound healing stages beyond inflammation is further evidenced by reports that directly treating dermal fibroblasts with platelets or platelet products such as platelet lysate [PL], platelet rich plasma [PRP] or platelet containing gels enhances wound healing responses [49-52].

Migration, invasion and MMP production

Upon tissue injury platelets synergies with the coagulation cascade to produce a fibrinogen matrix that can act as a temporary scaffold to allow leukocytes and fibroblasts to be recruited. This scaffold is rapidly degraded and replaced with a neo-ECM as migrating fibroblasts secrete collagens and fibronectins. Stimulating dermal fibroblasts with PL enhances *in vitro* scratch wound closure and alters the vimentin/actin cytoskeleton profile of these cells [53,54]. Vimentin levels correlate with a mesenchymal phenotype, decreased desmosome expression and enhanced motility [55]. In addition to enhancing motility, platelet products increase dermal fibroblast invasion [51,56-62], likely through the production of matrix metalloproteinases [MMPs]. MMPs are a group of zinc dependent protein hydrolases that break down components of the ECM and play a crucial role in tissue debridement and wound healing [63]. The overexpression of MMPs however is often reported in chronic wounds [64,65], where they degrade growth factors and destabilize the tissue matrix.

MMP-1 is a collagenase enzyme produced by dermal fibroblasts that is critical for wound healing through its ability to activate cytokines, loosen cell-ECM contacts and for collagen remodeling [66,67]. Active levels of the enzyme are increased

during re-epithelisation, and decline after wound closure [68]. Upon treatment with platelet products, dermal fibroblasts show enhanced expression of both MMP-1 mRNA and protein [57,58,69] and decreased levels of collagen-1 synthesis [69], suggesting that platelets may help regulate ECM remodeling. Similarly treatment of fibroblasts with platelet derived factors increases production of the gelatinases MMP-2 and MMP-9, [51] which are involved in granulation tissue remodelling for epithelisation [70,71]. Others have suggested treatment of dermal fibroblasts increases collagen production however, rather than reducing its expression levels [49,57,72-74]. These apparent discrepancies in matrix production/degradation may reflect the different models and animal types being used in individual studies, as well as the differing levels of cytokines in different platelet products.

Proliferation

PRP and releasate from platelets directly enhance dermal fibroblast proliferation [51,56-62] and metabolic activity [54]. The mitogenic and chemotactic potential of PL and PRP is likely due to the abundant source of growth factors and cytokines they contain, including platelet derived growth factor subtypes [PDGF-AB, BB, AA], TGF-β1 & 2, vascular endothelial growth factor [VEGF], epidermal growth factor [EGF], fibroblast growth factor [FGF-β], keratinocyte growth factor [KGF] as well as proinflammatory interferon [IFNγ] and TNF-α [51,56]. Both EGF and TGF-β stimulate fibroblast chemotaxis and proliferation [43,75,76]. PDGF is similarly a potent chemoattractant and mitogen, and also stimulates collagen synthesis [77]. PDGF promotes the migration and proliferation of murine myofibroblasts through the activation of p38 and extracellular signal regulated kinase [ERK] 1/2, respectively [78]. Similar pathways operate in human dermal fibroblasts treated with platelet contents, with wound closure reported to be strictly dependent on intracellular Ca^{2+}mobilisation and the activation of p38, ERK1/2 and phosphatidylinositol 3 kinase [PI3K] signaling [60]. The PI3K-Akt pathway and MAP kinases regulate cell cycle progression through modulating cyclin levels and cyclin dependent kinase activity [79-81]. In this respect, activated platelets induce DNA synthesis in dermal fibroblasts and PRP induces cell cycle progression through enhancing expression of key regulatory proteins including cyclin A, cyclin E and cyclin dependent kinase 4, [82,57]. A summary of the effects of platelets on dermal fibroblasts can be found in Figure 2.
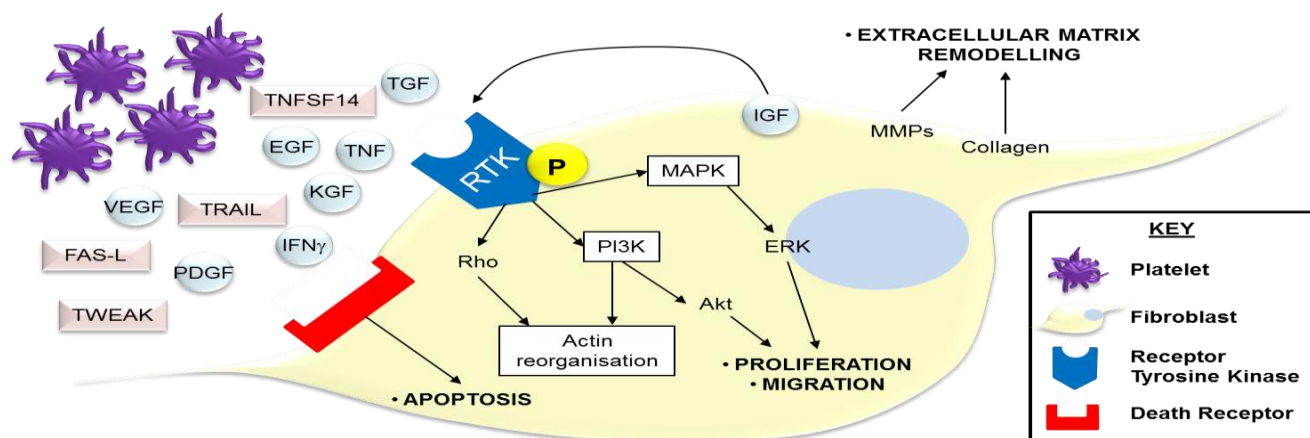
Figure 2: Platelet regulation of dermal fibroblasts. Activated platelets influence dermal fibroblast behavior in a number of ways, through the release of soluble cytokines, growth factors and apoptosis triggers. Please see main text for details. Abbreviations: EGF, epidermal growth factor; ERK, extracellular signal regulated kinase; FAS-L, Fas-ligand; IFN, interferon; IGF, insulin-like growth factor; KGF, keratinocyte growth factor; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; PDGF, platelet-derived growth factor; P13K, phosphotidylinositol-3-kinase; TGF, transforming growth factor; TNF, tumour necrosis factor; TNFSF14, TNF superfamily member 14; TRAIL, TNF-related apoptosis-inducing ligand; TWEAK, TNF-related weak inducer of apoptosis; VEGF, vascular endothelial growth factor.

Platelets, apoptosis and wound healing

Apoptosis is a major requirement for successful wound closure. During the inflammatory phase of wound healing neutrophils are initially recruited in large numbers to effectively decontaminate the area. Prolonged, elevated levels of neutrophils limit wound repair however [83], so must be quickly removed from injury sites, which largely occurs through macrophage induced apoptosis [84]. Indeed apoptosis of immune cells has been suggested to be the defining event signaling the transition from inflammation to wound resolution [85]. Additionally apoptosis of myo-fibroblasts and endothelial cells is required for granulation tissue to evolve into scar tissue, events which may be perturbed in diabetes leading to chronic wound development [86]. Platelets may help control the balance between cell survival and apoptosis, helping to determine the fate of injured tissue [87-89]. Activated platelet release a range of apoptotic signaling molecules including tumour necrosis factor superfamily 14 [TNFSFS14], TNF-related apoptosis-inducing ligand [TRAIL] and TNF-related weak inducer of apoptosis [TWEAK] [90], and express the death receptor ligand Fas-L on their surface [91,92], enabling them to induce apoptosis of murine embryonic fibroblasts (Figure 2). Platelets have previously been shown to induce endothelial cell apoptosis via caspase-8 and -9 [93]. Ligation of Fas activates pro-caspase 8, triggering the events allowing cytochrome c release from the mitochondria and ultimately controlled cell death [94]. Platelet aggregation to neutrophils, monocytes and lymphocytes has been reported to increase their rates of apoptosis in whole blood [95], suggesting platelet recruitment to damaged tissue may help control inflammation and granule tissue development through apoptosis.

Platelet stimulation of keratinocytes

There are comparatively few studies examining the effects of platelets and their releasate on keratinocyte physiology. Re-epithelisation is a key step of wound healing driven by the migration and proliferation of keratinocytes over the dermal surface of the wound bed. Initially keratinocytes lose their desmosome and hemidesmosome attachments before adopting an epithelial to mesenchymal transition [96]. As these migrating cells progress, keratinocytes at the wound margin proliferate to help create a new epidermal layer [43,75].

Proliferation and differentiation

Once differentiated, keratinocytes lose the ability to self-renew, and form new adherens junctions and desmosomes, resulting in a highly specialised, organized and stratified squamous epithelium [96]. This process is essential to maintain an effective external barrier. Terminal differentiation is preceded by alterations in intracellular protein such as keratin 1, 10, 14 [K1,10,14] and involucrin. Basal keratinocytes display K14 but not K10, whereas K14 positive K10 positive keratinocytes are in the transition from basal to a differentiated state, that will eventually become terminally differentiated non-proliferative K14 negative K10 positive cells. PRP treatment has been shown to keep keratinocytes in a K14 positive K10 negative phenotype, indicative of basal keratinocytes, but reduced overall proliferation [74]. It is likely that direct contact with fibroblasts is also needed to stimulate keratinocyte growth [97]. Others have similarly shown that platelet releasate does not promote [98], or may even diminish keratinocyte proliferation [72]. The absence of a mitogenic effect upon treatment with platelet released factors does not stop wound healing potential however. Denuded epithelium is initially covered by the migration of epithelial cells, while cell proliferation occurs later [98,99]. Platelets could therefore provide an initial motile signal for keratinocytes with fibroblasts then supporting proliferation to complete restoration of epithelial integrity, although this remains to be studied using co-culture systems.

Migration

PRP and PL have both been reported to increase keratinocyte [74] and keratinocyte cell line motility [98,100] and induce cytoskeletal rearrangement [98,100] indicative of a motile phenotype. Treatment of HaCaT keratinocytes with PL induces both syndecan-4 neo-synthesis and reorganisation [49]. Syndecan-4 is a transmembrane Heparan Sulphate proteoglycan important in the formation of focal adhesions and stress fibers [101]. It's essential role in cutaneous wound healing [102] stems from its ability to reorganise cell-matrix interactions and modulate integrin affinity [103,104]. A suggested PL-induced reorganisation of syndecan-4 could indicate integrin relocation

from hemi-desmosomes to focal adhesions is occurring, which preludes keratinocyte migration during re-epithelialisation [96,104]. The initiating signaling factors in PL driving the alteration to a more mobile phenotype are yet to be identified, although PDGF regulates syndecan-4 at both the translational and transcriptional level to induce fibroblast migration in wound healing [105]. PL induced keratinocyte migration has also been reported to be dependent on

Intracellular calcium mobilisation and p38 activation [98], mirroring PRP induced signaling events observed in fibroblasts. Platelet induce keratinocyte migration is likely to be further aided by the expression and activation of MMP-9 [49]. MMP-9 is a gelatinase/type-IV collagenase, both major components of the basement membrane. MMP-9 expression is particularly induced in epithelial cells at the front of the migrating epithelial sheet, where it aids in re-epithelisation [106]. A summary of the effect of platelets on keratinocytes can be found in Figure 3.
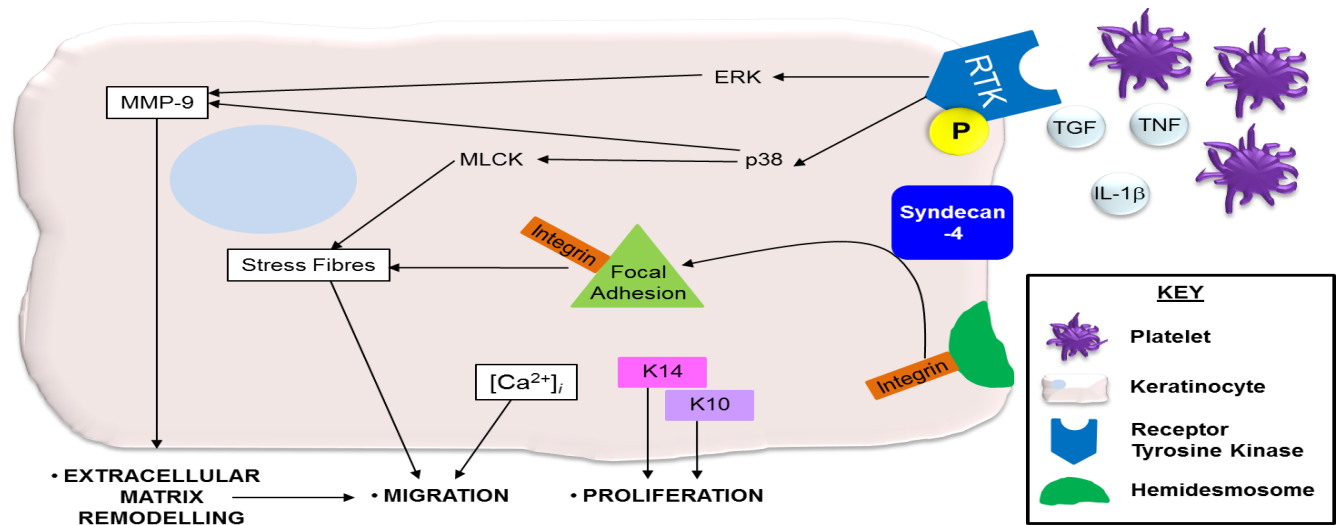
Figure 3: Platelet regulation of keratinocytes. Activated platelets modulate keratinocyte migration and proliferation through a number of known pathways, as well as by routes that are yet to be determined. Please see main text for details. Abbreviations: $[Ca^{2+}]_i$, intracellular calcium concentration; ERK, extracellular signal regulated kinase; IL-1β, interleukin-1 beta; K10 / K14, keratin 10 / 14; MLCK, myosin light chain kinase; MMP, matrix metalloproteinase; TGF, transforming growth factor; TNF, tumour necrosis factor.

Clinical use of platelet products in wound healing

Upon activation, platelets release a range of biologically active growth factors which promote inflammation, angiogenesis and tissue repair. Chronic wounds are characterized by a deficiency in these factors, which contributes to their delayed healing. Activated platelets therefore have the potential to release multiple, synergistic factors to re-start wound healing. The realisation that platelets and their associated contents integrate with all stages of tissue regeneration has led to platelet based products being used clinically to enhance wound healing for the past four decades. Principally these studies have used PRP [107-110] although some, generally earlier, studies have also focused on PL [111-113]. Unfortunately to date there is not a standardised procedure for PRP or PL preparation. Different

groups report variances in the volume of blood they aspirate, centrifuge, the anti-coagulant used, final platelet density achieved as well as differences in the levels and source of thrombin and Ca^{2+} added to gel the PRP. The volume, frequency and duration of PRP treatments also greatly differs between patients [114]. Altering the platelet concentration and activation status is likely to impact on the platelet degranulation profile, which could affect clinical outcomes [115]. These differences make it hard to draw overall objective conclusions about the effectiveness of topical treatment with platelet products, although there is a rapidly growing evidence base to establish their clinical relevance.

Randomised controlled trials [RCTs] remain the gold standard for determining treatment effect; however these studies often do not include patients with complex comorbidities who present

with a wide range of chronic wounds [116]. Small scale retrospective studies examining the ability of PRP to enhance closure of hard to heal wounds of different aetiologies have suggested a significant reduction in wound size with no adverse effects [117,118]. Similarly prospective studies on chronic wounds including pressure ulcers, venous ulcers and diabetic foot ulcers that had not responded to previous treatments, showed a significant decrease in mean wound area, suggesting that the application of PRP or related products can support cutaneous wound healing [119-121]. In a pilot open study examining the efficiency of a platelet gel preparation to heal skin ulcers in systemic sclerosis patients, Ferri et al. reported a significant reduction in wound size and increase in patient's quality of life as assessed by a health assessment questionnaire [122]. Platelet product usage has been suggested to be limited by the large volume of blood which sometimes must be aspirated from patients and the need for specialised equipment to extract the platelets. Additionally if using homologous platelets a suitable donor must be located and blood tested for a history of infectious disease. Kim et al. therefore tested the efficacy of using blood bank platelet concentrates in treating diabetic foot ulcers, reporting a significant effect on time required for complete healing and degree of wound shrinkage [123]. Controlled trials are now needed to confirm this clinical potency.

In 2011 a large observational multi-centre analysis of 200 chronic wound patients of mixed aetiologies showed a positive response in 96.5% of wounds treated with PRP with the authors reporting that rapid treatment response was observed in 275 of 285 wounds, and the magnitude of response was consistently high [124]. A previous multi-centered prospective, randomised, double-blinded controlled trial, that ultimately only included a small number of patients, suggested PRP gel-treated wounds are significantly more likely to heal than control gel treated wounds [125]. Ran et al. recently published the data from their prospective RCT examining the ability of autologous platelet-rich gel to facilitate the healing of diabetic chronic refractory cutaneous ulcers [126]. Analysis on a total of 117 diabetic ulcers randomised between the platelet gel treatment and control arms showed a significant difference in time to heal. It is generally accepted that insufficient growth factor levels and

poor host cell responses due to diabetes induce hyperglycaemia and vascular and neurological complications underpin the poor healing of diabetic chronic wounds. The concentrations of various growth factors in wounds increased after topical platelet product application [127,128], providing a possible explanation for the reported positive effects of PRP treatment although this correlation remains to be made.

Several systematic reviews have been undertaken to examine the clinical evidence for the use of PRP to enhance wound closure [129-132]. Garcia et al. analysed RCTs that together included 227 patients combined in the treated and control arms [125,133-136]. After assessing complete skin re-epithelisation in wound ulcers of mixed aetiologies, the pooled results showed PRP treatment had no significant effect above control. However a high degree of heterogeneity was found among the studies, with many was having methodological concerns. When study quality was graded for randomisation, method for double blinding and inclusion of a description of dropouts, only three studies were of high quality [125,133,134]. When these studies were analysed separately data were significantly favorable to PRP. The authors concluded the data for using PRP in wound healing is inconclusive however. Most studies were found to be small with varying methodologies, consequently strong, well-designed RCTs need to be developed to assess the efficacy and safety of PRP. Parnell et al. also noted significant variability in study quality for trials assessing PRP in wound healing [130]. They analysed twenty-four RCTs and comparative group studies using platelet rich plasma therapy for cutaneous wound healing, but found that PRP therapy significantly increased complete healing of chronic wounds and reduced the presence of infection in acute wounds. Similarly Gao et al. showed in the most recent systematic review that the use of PRP can shorten acute wound healing time, alleviate post-traumatic pain and may have some effect on the control of wound infections [131]. Wounds that are infected or heavily colonised do not heal as quickly as wounds that are not contaminated. Platelets are increasingly being ascribed a role in infection control mediated by their expression of toll-like receptors that recognise pathogen associated molecules, through their ability to be activated by bacterial lipopolysaccharides, and once activated their pre-disposition to release granular contents including an array of anti-microbial peptides and immunoregulatory molecules [137]. PRP has been

shown to have significant short term anti-bacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Streptococcus faecalis* [138]. The highly bactericidal actions of PRP [139] may prove useful in limiting the spread of bacteria prevalent within chronic wounds, contributing to the positive effects on wound closure reported, although this remains to be clinically determined.

CONCLUSION

Platelets play a strong supportive role in wound healing through regulating stages of inflammation and by directly signaling to fibroblasts and keratinocytes, aiding in motility and migration. Although the role of platelets in wound healing is now well described [4,140], there are comparatively few studies on platelet-induced changes in wounded skin at the molecular level. Therefore, key platelet-derived factors in terms of expression and functionality need to be characterized in order to better understand their role in the healing process. Given the promising outcomes of treating wounded tissue with platelet products, advances in this field may help us to design new therapies for chronic wounds, which are becoming a growing issue as the population ages and incidence of diabetes increases.

REFERENCES

1.   C. Gonzalez, T. F. Costa, Z. A. Andrade, A. R. Medrado., *An. Bras. Dermatol*. **2016**, 91(5), 614-20.

2.   K. M. Naseem, W. Roberts., *Platelets*. **2011**, 22(2), 148-52.

3.   P. Blair, R. Flaumenhaft., *Blood. Rev*. **2009**, 23(4), 177-89.

4.   N. Katoh., *J. Derm. Sci*. **2009**, 53(2), 89-95.

5.   D. Projahn, R. R. Koenen., *J. Leuk. Bio*. **2012**, 92(6), 1167-75.

6.   M. Berger, J. A. Gray, B. L. Roth., *An. Rev. Med*. **2009**, 60, 355-66.

7.   Rowley JW, Schwertz H, A. S. Weyrich., *Curr. Opin. Hematol*. **2012**, 19(5), 385-91.

8.   A. Zarbock, R. K. Polanowska-Grabowska, K. Ley., *Blood. Rev*. **2007**, 21(2), 99-111.

9.   D. D. Wagner, P. S. Frenette., *Blood*. **2008**, 111(11), 5271-81.

10. P. Von Hundelshausen, R. R. Koenen, C. Weber., *Microcir*. **2009**, 16(1), 84-96.

11. F. W. Lam, A. R. Burns, C. W. Smith, R. E. Rumbaut., *Ame. J. Physio. Hea. Circu. Physio*. **2011**, 300(2), H468-75.

12. D. Duerschmied, G. L. Suidan, M. Demers, N. Herr, C. Carbo, A. Brill., *Blood*. **2013**, 121(6), 1008-15.

13. H. Tomita, Y. Iwata, F. Ogawa, K. Komura, K. Shimizu, A. Yoshizaki., *The. J. Invest. Derma*. **2009**, 129(8), 2059-67.

14. R. Elgueta, M. J. Benson, V. C. de Vries, A. Wasiuk, Y. Guo, R. J. Noelle., *Immun. Rev*. **2009**, 229(1), 152-72.

15. V. Henn, J. R. Slupsky, M. Grafe, I. Anagnostopoulos, R. Forster, G. Muller-Berghaus., *Nat*. **1998**, 391(6667), 591-4.

16. H. Mysliwiec, I. Flisiak, A. Baran, M. Gorska, B. Chodynicka., Folia. Histochem. *Cytobiol*. **2012**, 50(1), 75-9.

17. M. C. Pasch, K. K. Timar, M. van Meurs, V. M. Heydendael, J. D. Bos, JD Laman., *The. J. Patho*. **2004**, 203(3), 839-48.

18. Companjen AR, van der Wel LI, Boon L, Prens EP, Laman JD., *Int. Immunol*. **2002**, 14(6), 669-76.

19. R. Tamagawa-Mineoka, N. Katoh, S. Kishimoto., *J. Am. Acad. Dermatol*. **2010**, 62(4), 621-6.

20. R. Tamagawa-Mineoka, N. Katoh, E. Ueda, K. Masuda, S. Kishimoto., *Allergol. Int*. **2008**, 57(4), 391-6.

21. R. Tamagawa-Mineoka., *J. Dermato. Sci*. **2015**, 77(2), 93-101.

22. T. Kato, H. Saeki, Y. Tsunemi, S. Shibata, K. Tamaki, S. Sato., *Exp. Dermatol*. **2011**, 20(8), 669-74.

23. T. Fujisawa, R. Fujisawa, Y. Kato, T. Nakayama, A. Morita, H. Katsumata., *The. J. Alle. Clin. Immuno*. **2002**, 110(1), 139-46.

24. P. von Hundelshausen, C. Weber., *Cir. Res*. **2007**, 100(1), 27-40.

25. Kasper, E. Brandt, S. Bulfone-Paus, F. Petersen., *Blood*. **2004**, 103(5), 1602-10.

26.   P. von Hundelshausen, R. R. Koenen, M. Sack, S. F. Mause, W. Adriaens, A. E. Proudfoot., *Blood*. **2005**, 105(3), 924-30.

27.   P. von Hundelshausen, K. S. Weber, Y. Huo, A. E. Proudfoot, P. J. Nelson, K. Ley., *Cir*. **2001**, 103(13), 1772-7.

28.   A. Schobe, D. Manka, P. von Hundelshausen, Y. Huo, P. Hanrath, I. J. Sarembock., *Cir*. **2002**, 106(12), 1523-9.

29.   N. Gerdes, L. Zhu, A. Hermansson, P. Hjemdahl, H. Hu., *Throm. Haemo*. **2011**, 106(2), 353-62.

30.   W. Ouyang, J. K. Kolls, Y. Zheng., *Immu*. **2008**, 28(4), 454-67.

31.   S. Lindemann, N. D. Tolley, J. R. Eyre, L. W. Kraiss, T. M. Mahoney, A. S. Weyrich., *The. J. Bio. Chem*. **2001**, 276(36), 33947-51.

32.   M. G. Netea, F. L. van de Veerdonk, J. W. van der Meer, C. A. Dinarello, L. A. Joosten., An. Rev. Immuno. **2015**, 33, 49-77.

33.   S. A. Eming, T. Krieg, J. M. Davidson., *J. Invest. Derma*. **2007**, 127(3), 514-25.

34.   Y. Ishida, T. Kondo, A. Kimura, K. Matsushima, N. Mukaida., *J. Immuno*. **2006**, 176(9), 5598-606.

35.   R. E. Mirza, M. M. Fang, W. J. Ennis, T. J. Koh., *Diab*. **2013**, 62(7), 2579-87.

36.   Gong, W. Shi, S. J. Yi, H. Chen, J. Groffen, N. Heisterkamp., *B. M. C. Immuno*. **2012**, 13, 31.

37.   M. Mosser, J. P. Edwards., *Nat. Rev. Immuno*. **2008**, 8(12), 958-69.

38.   O. Martinez, L. Helming, S. Gordon., *Ann. Rev. Immuno*. **2009**, 27, 451-83.

39.   X. J. Wang, G. Han, P. Owens, Y. Siddiqui, A. G. Li., *J. Investig. Dermatol. Symp*. **2006**, 11(1), 112-7.

40.   M. Pakyari, A. Farrokhi, M. K. Maharlooei., *Advan. Wou. Care*. **2013**, 2(5), 215-24.

41.   Y. Vodovotz, C. Bogdan, J. Paik, Q. W. Xie, C. Nathan., *The. J. Experimen. Med*. **1993**, 178(2), 605-13.

42.   A. Soneja, M. Drews, T. Malinski., *Pharmacol. Rep*. **2005**, 57 Suppl, 108-19.

43.   S. Barrientos, O. Stojadinovic, M. S. Golinko, H. Brem, M. Tomic-Canic., Wound Healing Society (and) the European Tissue Repair Society. **2008**, 16(5), 585-601.

44.   B. Jude, R. Blakytny, J. Bulmer, A. J. Boulton, M. W. Ferguson., *Diab. Med. A. J. Brit. Diab. Asso*. **2002**, 19(6), 440-7.

45.   P. Schmid, D. Cox, G. Bilbe, G. McMaster, C. Morrison, H. Stahelin., *The. J. Path*. **1993**, 171(3), 191-7.

46.   Pastar, O. Stojadinovic, A. Krzyzanowska, S. Barrientos, C. Stuelten, K. Zimmerman., *Mol. Med*. **2010**, 16(3-4), 92-101.

47.   C. Deppermann, D. Cherpokova, P. Nurden, J. N. Schulz, I. Thielmann, P. Kraft., *The. J. Clin. Investi*. **2013**.

48.   C. Deppermann, P. Nurden, A. T. Nurden, B. Nieswandt, D. Stegner., *Ra. Dis*. **2013**, 1, e26561.

49.   E. Ranzato, S. Martinotti, A. Volante, L. Mazzucco, B. Burlando., *Exp. Dermatol*. **2011**, 20(4), 308-13.

50.   L. J. Xian, S. R. Chowdhury, A. Bin Saim, R. B. Idrus., *Cytother*. **2015**, 17(3), 293-300.

51.   Giusti, A. Rughetti, S. D'Ascenzo, G. Di Stefano, M. R. Nanni, D. Millimaggi., Wound Healing Society (and) the European Tissue Repair Society. **2013**, 21(2), 300-8.

52.   V. N. Obolenskiy, D. A. Ermolova, L. A. Laberko., *Wou. Med*. **2017**, 19, 27-32.

53.   M. C. arsotti, P. Losi, E. Briganti, E. Sanguinetti, A. Magera, T. Al Kayal., *PloS One*. **2013**, 8(12), e84753.

54.   V. Cipriani, E. Ranzato, V. Balbo, L. Mazzucco, M. Cavaletto, M. Patrone., *J. Tis. Eng. Regen. Med*. **2009**, 3(7), 531-8.

55.   M. G. Mendez, S. Kojima, R. D. Goldman., *F. A. S. E. B. J*. **2010**, 24(6), 1838-51.

56.   R. Lundquist, K. Holmstrom, C. Clausen, B. Jorgensen, T. Karlsmark., Wound repair and regeneration. **2013**, 21(1), 66-76.

57.   W. Cho, S. A. Kim, K. S. Lee., *Int. J. Mol. Med*. **2012**, 29(1), 32-6.

58.   Bernuzzi, S. Tardito, O. Bussolati, D. Adorni, S. Cantarelli, F. Fagnoni., *Blood. Trans. Trasf. Del. Sangue*. **2010**, 8(4), 237-47.

59.   M. Ruangchainikom, S. Waikakul, A. Wongkajornsilp, V. Chongkolwatana., *J. Orthop. Surg*. **2009**, 17(2), 202-5.

60.   E. Ranzato, L. Mazzucco, M. Patrone, B. Burlando., *J. Cel. Mol. Med*. **2009**, 13(8B), 2030-8.

61.   E. Anitua, M. Sanchez, M. W. Zalduendo, M. de la Fuente, R. Prado, G. Orive., *Cel. Prol*. **2009**, 42(2), 162-70.

62.   N. Kakudo, T. Minakata, T. Mitsui, S. Kushida, F. Z. Notodihardjo, K. Kusumoto., *Plas. Recons. Surg*. **2008**, 122(5), 1352-60.

63.   M. P. Caley, V. L. Martins, E. A. O'Toole., *Adva. Wou. Care*. **2015**, 4(4), 225-34.

64.   N. J. Trengove, M. C. Stacey, S. MacAuley, N. Bennett, J. Gibson, F. Burslem., *Wou. Rep. Regen*. **1999,** 7(6), 442-52.

65.   D. R. Yager, L. Y. Zhang, H. X. Liang, R. F. Diegelmann, I. K. Cohen., *The. J. Invest. Dermat*. **1996**, 107(5), 743-8.

66.   B. K. Pilcher, J. A. Dumin, B. D. Sudbeck, S. M. Krane, H. G. Welgus, W. C. Parks., *J. Cel. Biol*. **1997**, 137(6), 1445-57.

67.   S. E. Gill, W. C. Parks., *Int. J. Biochem. Cel. Biol*. **2008**, 40(6-7), 1334-47.

68.   Q. Gu, D. Wang, Y. Gao, J. Zhou, R. Peng, Y. Cui., *J. Environ. Path. Toxic. Onco*. **2002**, 21(1), 71-8.

69.   M. K. Shin, J. W. Lee, Y. I. Kim, Y. O. Kim, H. Seok, N. I. Kim., *Mol. Bio. Rep.* **2014**, 41(1), 3-8.

70.   M. Makela, H. Larjava, E. Pirila, P. Maisi, T. Salo, T. Sorsa., *Exper. Cel. Res*. **1999**, 251(1), 67-78.

71.   T. Salo, T, Makela M, Kylmaniemi M, Autio-Harmainen H, Larjava H., *Lab. Invest*. 1994, 70(2), 176-82.

72.   H. Wang, Y. P. Wu, W. C. Lo., *Rejuven. Res.* **2012**, 15(6), 613-23.

73.   K. Shin, J. H. Lee, S. J. Lee, N. I. Kim., Derma. *Surg*. **2012**, 38(4), 623-30.

74.   J. Xian, S. Roy Chowdhury, A. Bin Saim, R. Idrus., *Cytother*. **2015**, 17(3), 293-300.

75.   S. Werner, R. Grose., *Physio. Rev*. **2003**, 83(3), 835-70.

76.   Hetzel, M. Bachem, D. Anders, G. Trischler, M. Faehling., *Lung**. **2005**, 183(4), 225-37.

77.   R. A. Clark., *The. Amer. J. Med. Sci*. **1993**, 306(1), 42-8.

78.   Tangkijvanich, C. Santiskulvong, A. C. Melton, E. Rozengurt, H. F. Yee, *Jr. J. Cel. Physio*. **2002**, 191(3), 351-61.

79.   W. Zhang, H. T. Liu., *Cel. Res*. **2002**, 12(1), 9-18.

80.   Liang, J. M. Slingerland., *Cel. Cyc*. **2003**, 2(4), 339-45.

81.   M. A. Stull, A. M. Rowzee, A. V. Loladze, T. L. Wood., *J. Mam. Gla. Bio. Neopla*. **2004**, 9(1), 15-26.

82.   F. Giacco, G. Perruolo, E. D'Agostino, G. Fratellanza, E. Perna, S. Misso., *F. A. S. E. B. J.* **2006**, 20(13), 2402-4.

83.   V. Dovi, A. M. Szpaderska, L. A. DiPietro., *Thromb. Haemos*. **2004**, 92(2), 275-80.

84.   A. J. Meszaros, J. S. Reichner, J. E. Albina., *J. Immuno*. **2000**, 165(1), 435-41.

85.   Y. S. Wu, S. N. Chen., *Fro. Pharma*. **2014**, 5, 1.

86.   A. K. Arya, R. Tripathi, S. Kumar, K. Tripathi., *Wor. J. Dia.* **2014**, 5(6), 756-62.

87.   Sharron, C. E. Hoptay, A. A. Wiles, L. M. Garvin, M. Geha, A. S. Benton., *PloS. One*. **2012**, 7(7), e41549.

88.   H. Gambim, O. do Carmo Ade, L. Marti, S. Verissimo-Filho, L. R. Lopes, M. Janiszewski., *Crit. Care*. **2007**, 11(5), R107.

89.   Janiszewski, A. O. Do Carmo, M. A. Pedro, E. Silva, E. Knobel, F. R. Laurindo., *Crit. Care. Med*. **2004**, 32(3), 818-25.

90.   M. Gawaz, S. Vogel., *Blood*. **2013**, 122(15), 2550-4.

91.   R. I. Schleicher, F. Reichenbach, P. Kraft, A. Kumar, M. Lescan, F. Todt., *Blood*. **2015**, 126(12), 1483-93.

92.   R. Ahmad, J. Menezes, L. Knafo, A. Ahmad., *J. Leuko. Bio*. **2001**, 69(1), 123-8.

93.   C. J. Kuckleburg, R. Tiwari, C. J. Czuprynski., *Throm. Haemos*. **2008**, 99(2), 363-72.

94.   M. Kruidering, G. I. Evan., *I. U. B. M. B. Life*. **2000**, 50(2), 85-90.

95.   S. Hartz, B. Menart, D. Tschoepe., *Cytomet. A*. **2003**, 52(2), 117-21.

96.   M. M. Santoro, G. Gaudino., *Experimen. Cel. Res*. **2005,** 304(1), 274-86.

97.   Z. Wang, Y. Wang, F. Farhangfar, M. Zimmer, Y. Zhang., *PloS. One*. **2012**, 7(7), e40951.

98.   E. Ranzato, M. Patrone, L. Mazzucco, B. Burlando., *Brazil. J. Dermatol*. **2008**, 159(3), 537-45.

99.   Y. Matsubayashi, M. Ebisuya, S. Honjoh, E. Nishida., *Curr. Biol.* **2004**, 14(8), 731-5. R. El.

100.   Backly, V. Ulivi, L. Tonachini, R. Cancedda, F. Descalzi, M. Mastrogiacomo., *Tissue. Eng.* **2011**, 17(13-14), 1787-800.

101.   A. Woods, R. L. Longley, S. Tumova, J. R. Couchman., *Arch. Biochem. Biophys.* **2000**, 374(1), 66-72.

102.   F. Echtermeyer, M. Streit, S. Wilcox-Adelman, S. Saoncella, F. Denhez, M. Detma. *The. J. Clin. Invest.* **2001**, 107(2), R9-R14.

103.   S. Munesue, Y. Kusano, K. Oguri, N. Itano, Y. Yoshitomi, H. Nakanishi., *Biochem. J.* **2002**, 363(Pt 2), 201-9.

104.   E. Araki, Y. Momota, T. Togo, M. Tanioka, K. Hozumi, M. Nomizu., *Mol. Biol. Cell.* **2009**, 20(13), 3012-24.

105.   F. Lin, X. D. Ren, G. Doris, R. A. Clark., *The. J. Invest. Derma.* **2005**, 124(5), 906-13.

106.   R. Mohan, S. K. Chintala, J. C. Jung, W. V. Villar, F. McCabe, L. A. Russo., *The. J. Biol. Chem.* **2002**, 277(3), 2065-72.

107.   Borzini, L. Mazzucco., *Curr. Opin. Hematol.* **2005**, 12(6), 473-9.

108.   Crovetti, G. Martinelli, M. Issi, M. Barone, M. Guizzardi, B. Campanati., *Transfus. Apher. Sci.* **2004**, 30(2), 145-51.

109.   D. M. Dohan Ehrenfest, L. Rasmusson, T. Albrektsson., *Tren. Biotechnol.* **2009**, 27(3), 158-67.

110.   L. Mazzucco, D. Medici, M. Serra, R. Panizza, G. Rivara, S. Orecchia., *Transf.* **2004,** 44(7), 1013-8.

111.   D. R. Knighton, K. F. Ciresi, V. D. Fiegel, L. L. Austin, E. L. Butler., *Ann. Surg.* **1986**, 204(3), 322-30.

112.   S. C. Atri, J. Misra, D. Bisht, K. Misra., *Surg.* **1990**, 108(3), 508-12.

113.   D. L. Steed, J. B. Goslen, G. A. Holloway, J. M. Malone, T. J. Bunt., *Diab. Care.* **1992**, 15(11), 1598-604.

114.   L. Mazzucco, P. Borzini, R. Gope., *Transfus. Med. Rev.* **2010**, 24(3), 218-34.

115.   R. S. Dhillon, E. M. Schwarz, M. D. Maloney., *Arthri. Res. Ther.* **2012**, 14(4), 219.

116.   M. J. Carter, C. E. Fife, D. Walker, B. Thomson., *Adv. Skin. Wou. Care.* **2009**, 22(7), 316-24.

117.   Steenvoorde, L. P. van Doorn, C. Naves, J. Oskam., *J. Wou. Care.* **2008**, 17(2), 60-3.

118.   Sakata, S. Sasaki, K. Handa, T. Uchino, T. Sasaki, R. Higashita., *Ostomy. Wou. Manage.* **2012**, 58(4), 36-49.

119.   G. Frykberg, V. R. Driver, D. Carman, B. Lucero, C. Borris-Hale, C. P. Fylling., Ostomy. Wou. Manage. **2010**, 56(6), 36-44.

120.   B. Jorgensen, T. Karlsmark, H. Vogensen, L. Haase, R. Lundquist., *Int. J. Lo. Extrem. Woun.* **2011**, 10(4), 218-23.

121.   M. O'Connell, T. Impeduglia, K. Hessler, X. J. Wang, R. J. Carroll, H. Dardik., *Wou. Rep. Reg.* **2008**, 16(6), 749-56.

122.   D. Giuggioli, M. Colaci, A. Manfredi, M. Mariano, C. Ferri., *Rheumatol. Int.* **2012**, 32(9), 2929-32.

123.   H. Jeong, S. K. Han, W. K. Kim., *Plas. Recon. Surg.* **2010**, 125(3), 944-52.

124.   M. de Leon, V. R. Driver, C. P. Fylling, M. J. Carter, C. Anderson, J. Wilson., *Adv. Skin. Wou. Care.* **2011**, 24(8), 357-68.

125.   V. R. Driver, J. Hanft, C. P. Fylling, J. M. Beriou., *Ostomy. Wou. Manage.* **2006**, 52(6), 68-70, 2, 4 passim.

126.   Li, D. Chen, C. Wang, N. Yuan, Y. Wang, L. He., *Wou. Rep. Rege.* **2015**, 23(4), 495-505.

127.   G. Pietramaggiori, S. S. Scherer, J. C. Mathews, M. Alperovich, H. J. Yang, J. Neuwalder., *Wou. Rep. Rege.* **2008**, 16(2), 218-25.

128.   P. He, C. Wang, D. W. Chen, X. J. Li., (Dynamic changes of MMP-1, MMP-9 and TIMP-1 in the refractory diabetic dermal ulcers treated by autologous Platelet-rich gel). Sichuan Da Xue Xue Bao Yi Xue Ban. **2012**, 43(5), 757-61.

129.   J. Martinez-Zapata, A. Marti-Carvajal, I. Sola, I. Bolibar, J. Angel Exposito, L. Rodriguez., *Transfu.* **2009**, 49(1), 44-56.

130.   J. Carter, C. P. Fylling, L. K. Parnell., *Eplasty.* **2011**, 11, e38.

131.   L. Wang, Z. Gu, C. Gao., (Platelet-rich plasma for treating acute wounds, a meta-analysis) Zhonghua Yi Xue Za Zhi. **2014**, 94(28), 2169-74.

132.   D. L. Villela, V. L. Santos., *Grow. Fact.* **2010**, 28(2), 111-6.

133.   D. R. Knighton, K. Ciresi, V. D. Fiegel, S. Schumerth, E. Butler, F. Cerra., *Surg. Gynecol. Obstet.* **1990**, 170(1), 56-60.

134.   W. C. Krupski, L. M. Reilly, S. Perez, K. M. Moss, P. A. Crombleholme, J. H. Rapp., *J. Vasc. Surg*. **1991**, 14(4), 526-32.

135.   M. C. Stacey, S. D. Mata, N. J. Trengove, C. A. Mather., *Eur. J. Vasc. Endovasc. Surg*. **2000**, 20(3), 296-301.

136.   Senet, F. X. Bon, M. Benbunan, A. Bussel, R. Traineau, F. Calvo., *J. Vasc. Surg*. **2003**, 38(6), 1342-8.

137.   K. J. Clemetson., *Hamostaseologie*. **2011**, 31(4), 264-8.

138.   E. Mariani, G. Filardo, V. Canella, A. Berlingeri, A. Bielli, L. Cattini., *Cytotherapy*. **2014**, 16(9), 1294-304.

139.   C. M. Edelblute, A. L. Donate, B. Y. Hargrave, L. C. Heller., *Platelates*. **2015**, 26(1), 13-6.

140.   M. Chiara Barsotti, P. Losi, E. Briganti, E. Sanguinetti, A. Magera, T. Al Kayal., *PloS. One*. **2013**, 8(12), e84753.