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ORIGINAL ARTICLE

The use of fat grafting and platelet-rich plasma for wound healing: A review of the current evidence

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Fat grafting is becoming a common procedure in regenerative medicine because of its high content of growth factors and adipose derived stem cells (ADSCs) and the ease of harvest, safety, and low cost. The high concentration of ADSCs found in fat has the potential to differentiate into a wide range of wound-healing cells including fibroblasts and keratinocytes as well as demonstrating proangiogenic qualities. This suggests that fat could play an important role in wound healing. However retention rates of fat grafts are highly variable due in part to inconsistent vascularisation of the transplanted fat. Furthermore, conditions such as diabetes, which have a high prevalence of chronic wounds, reduce the potency and regenerative potential of ADSCs. Platelet-rich plasma (PRP) is an autologous blood product rich in growth factors, cell adhesion molecules, and cytokines. It has been hypothesised that PRP may have a positive effect on the survival and retention of fat grafts because of improved proliferation and differentiations of ADSCs, reduced inflammation, and improved vascularisation. There is also increasing interest in a possible synergistic effect that PRP may have on the healing potential of fat, although the evidence for this is very limited. In this review, we evaluate the evidence in both in vitro and animal studies on the mechanistic relationship between fat and PRP and how this translates to a benefit in wound healing. We also discuss future directions for both research and clinical practice on how to enhance the regenerative potential of the combination of PRP and fat.

KEYWORDS
adipose-derived stem cells, fat grafting, platelet-rich plasma, wound healing

1 | INTRODUCTION

Autologous fat grafting is a popular procedure in plastic and reconstructive surgery because of its versatility, ease of harvesting, and low donor site morbidity. It is used extensively for the contouring of soft tissue defects[1] but recent studies have shown its versatility for a wider variety of purposes including the softening of scars[2] and the improvement of fibrosis in scleroderma.[3] More recently, there has been significant interest in the regenerative potential of autologous fat.[4,5] This is because the ease of harvest offers a cheap, safe, and direct route to an abundant population of adipose-derived stem cells (ADSCs) found within fat. These multipotent precursor cells are able to differentiate into cell lineages associated with the regeneration of tissues such as fibroblasts, keratinocytes, and endothelial cells.[6] Also found within the fat extracellular matrix, and secreted from the ADSCs themselves, are prohealing growth factors, anti-inflammatory cytokines, proangiogenic factors, and healing-related peptides[7,8] which may also have a positive effect on the healing process. Several clinical studies have found a potential benefit for the use of fat in the improvement of burn scars,[9] osteoarthritis,[10] and chronic radiotherapy scarring.[11] There is also some evidence to suggest a benefit of fat grafting in the healing of chronic and acute wounds,[12–14] although the quality of evidence is limited with no prospective randomised controlled trials. Furthermore, fat may have a role in wound healing of conditions such as diabetes (which cause reduced peripheral vascularisation leading to

chronic wounds) as ADSCs may have a potent effect on angiogenesis and restoration of blood flow.[15]

However, the long-term retention rate of fat grafting is highly variable, with up to 80% loss of graft reported in some studies,[16–18] limiting its potential use in chronic wound healing. Theories regarding why fat grafts fail include technical factors in the preparation of fat,[19,20] local wound infection, and patient factors such as age, body mass index, and diabetes.[21] These factors alone are often associated with poor wound healing, although they may be correctable through a careful and meticulous technique or appropriate patient selection. However, inadequate neovascularisation of the transplanted fat has also been hypothesised as a significant factor in the failure of fat grafting. When fat is injected, the mechanical pressure may cause damage and ischaemic injury to the fat immediately reducing its vascularity.[22] For adipogenesis to occur, there must be adequate early angiogenesis and thus adipocytes and ADSCs are known to be poorly tolerant of ischaemic conditions.[23] Histological evaluation of injected fat illustrates necrosis of adipocytes when a delay in establishing blood supply occurs.[24] Fat grafts that are well vascularised illustrate improved retention rates in experimental conditions; [25,26] however, it is very difficult to control for this in the clinical setting without an adjunctive agent.

Therefore, several autologous products have been trialled in combination with fat to improve its retention rate through the improvement of neovascularisation, including stromal vascular fraction (SVF)[27] and platelet-rich fibrin.[28] However, the evidence for these procedures is extremely limited. By far, the most commonly combined autologous tissue with fat in the literature is platelet-rich plasma (PRP). PRP is an autologous blood product rich in growth factors and cytokines.[29,30] When used as an isolated treatment, it has been shown in some studies to have beneficial effects for a wide variety of regenerative purposes including wound healing,[31] burn healing,[32] alopecia,[33] osteoarthritis,[34] tendon healing,[35] and nerve regeneration.[36] However, its efficacy as a single treatment for wound healing has not been proven with higher level evidence showing no overall benefit.[37] This may be because of wide variation in PRP preparation methodology leading to an unreliable and non-reproducible growth factor content.[38]

PRP contains an abundance of growth factors, proangiogenic factors, and cell adhesion molecules, which may significantly enhance the survival of fat cells through improvement in proliferation, differentiation, and angiogenesis.[39] Furthermore, when PRP is used in combination with fat there may be a synergistic effect on the regenerative potential of both treatments as both have significant stores of prohealing factors.[40] Given the ease of harvesting, the low donor site morbidity of both products, and the straightforward mixing methodology, a combination treatment provides an exciting prospect in wound healing. However, despite this, clinical application for combined fat/PRP in wound healing has been very limited with only three human studies thus far, the conclusions of which are very limited by small patient numbers and lack of high-quality methodology.[41]

Key Messages

- fat grafting and platelet-rich plasma when used in combination have significant potential to improve wound healing
- the aim of this manuscript was to discuss the theory regarding this technique and to evaluate the current evidence from in vitro and animal studies to better understand the potential of this technique
- although the theory makes sense scientifically, there have been very few studies directly assessing why a combination of these two autologous treatments benefits the healing process
- evidence of in vitro and animal studies directly evaluating efficacy is extremely limited and further animal mode studies and clinical randomised controlled studies are required

Therefore, the aim of this review is to discuss and synthesise all of the current available evidence on the regenerative wound-healing potential of the combination treatment of fat and PRP and the theory behind this. This study discusses in vitro and animal studies and suggests potential developments for future research in wound healing. It does not discuss the methodology for preparation of either fat or PRP nor does it discuss the different methods of mixing the two, as reviewed in a previous article.[42]

2 | THE WOUND-HEALING PROCESS

To understand how fat and PRP in combination can have an effect on wound healing, the process of healing must be summarised. Wound healing is a process that results in the restoration of normal architecture and function of the damaged tissue through a physiological process. There are four phases of wound healing: haemostasis; inflammation; proliferation; and remodelling.[43] Haemostasis occurs immediately after tissue damage and results in the formation of a platelet plug that adheres to damaged endothelial surfaces of blood vessels. Strands of fibrin reinforce the plug forming a thrombus that releases growth factors and forms a scaffold for migrating cells. During the inflammatory stage, neutrophils and monocytes are recruited to the site and differentiate to macrophages, which remove debris, damaged cells, and pathogens from the wound to prevent infection. Blood vessels dilate allowing the migration of healing cells along with growth factors, nutrients, and antibodies causing oedema and erythema at the site. After approximately a week, the proliferation phase begins, which is characterised by new tissue formation with the migration, differentiation, and proliferation of prohealing cells. The fibrin matrix of the

haemostatic clot is replaced with granulation tissue by fibroblasts with the deposition of type 3 collagen and the extracellular matrix, which provides a nutrient supply and scaffold for angiogenesis. Remodelling and maturation of the granulation tissue then occur over a longer period to produce a mature scar. In order for a wound to heal effectively, there are several crucial factors but of particular importance are: a short inflammatory stage and quick transition to the proliferative stage,[44] and the adequate supply of oxygen and nutrients. These can be affected by a wide range of local (eg, infection, ischaemia, and foreign bodies) and systemic factors (eg, age, immunosuppression, and diabetes). Enhancing the factors that positively effect healing and inhibiting the negative factors is the theory regarding the potentially beneficial effects of PRP and fat and we will now discuss the evidence regarding this.

3 | PLATELET-RICH PLASMA

PRP is obtained via the centrifugation of whole blood that is obtained via peripheral venipuncture and mixed with acid-citrate dextrose (ACDA). There are a wide range of commercially available kits that generate PRP from whole blood, all of which have slightly differing methodologies and compositions. Once PRP is generated, it may or may not be "activated" to allow the release of alpha granules using the addition of a variety of compounds including calcium chloride, calcium gluconate, or thombin. The evidence for the benefit of one PRP device or one activation method over the other is very limited with no clearly standardised protocol in the literature.[31,45] This heterogeneity can lead to evidence that is often contradictory or difficult to compare.

PRP is defined as a platelet concentration above the normal platelet count of a defined volume of plasma.[46] However, a concentration of over approximately 1 million platelets per microlitre is thought to be clinically beneficial.[46,47] Alpha granules contained within platelets contain many growth factors that are known to be prohealing including platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor β (TGF-β), insulin-like growth factor (IGF), fibroblast growth factor (FGF), and epithelial growth factor (EGF).[48] The granules also secrete cytokines that are involved in cell migration and growth factor release, and proinflammatory molecules such as serotonin and histamine, which encourage the inflammation stage of wound healing.[30]

4 | FAT GRAFTING

Standard clinical fat grafting as described by Coleman involves the harvesting of fat from a defined donor site (abdomen, thigh, etc.) using negative pressure to extract the fat via cannulas. The fat is then centrifuged to allow separation of the lipoaspirate from the supernatant (oil layer) and infranatant (blood, water, and aqueous solution).[1] The lipoaspirate is the tissue that is grafted and contains two principle components that are not separated before conventional grafting: mature adipocytes and SVF. Further centrifugation of the lipoaspirate would produce a cell-dense pellet within the lower layer, which would contain the SVF and ADSCs that could be further isolated in the laboratory. Many in vitro and animal studies have evaluated the potential therapeutic benefit of isolating ADSCs and although there may be a beneficial effect in wound healing,[49–53] the clinical application of ADSCs is extremely limited in clinical practice because of cost, logistical issues, and ethical concerns[54] with governmental restrictions in place in many countries including the USA.

The SVF of the fat graft contains preadipocytes and multipotent ADSCs, which have the potential to differentiate into wound-healing cells such as fibroblasts, endothelial cells, and keratinocytes.[6,55] ADSCs have been shown to encourage neovascularisation and tissue regeneration in vivo.[52,56] Adipose tissue has one of the highest concentrations of stem cells in the body, with approximately 5000 ADSCs per gram of fat.[57] With regard to wound healing, the ADSCs may contribute to anti-inflammatory and proangiogenic function through the paracrine secretion of soluble mediators.[58] The SVF also contains haematopoeitic-lineage cells, mature endothelial cells, pericytes, fibroblasts, and white blood cells all of which have a role in wound healing.[59] Cultured ADSCs have also been shown to differentiate into vascular endothelial cells and then capillary structures indicating a potentially important role in the angiogenesis stage of wound healing.[60,61]

However, the main limitation of fat grafting is inconsistent survival of the graft,[62] which may limit its effectiveness in wound healing given most wounds take several weeks to heal. The retention of the fat may be directly related to the volume grafted[63] with the deepest adipocytes the most prone to cell death but it is also related to inadequate vascularisation of the transplanted fat tissue. Mature adipocytes are highly sensitive to ischaemia and are prone to early cell death without adequate oxygenation;[23] however, they may recover if adequate vascular supply can be established early after grafting.[64] It is reasonable to hypothesise that a fat graft that survives for longer would have an improved healing outcome as it would continue to release prohealing mediators as well as allowing ADSCs to differentiate for longer.

5 | THE ROLE OF PRP IN FAT GRAFT SURVIVAL

There have been several clinical studies that have shown a beneficial effect of PRP on the outcome of fat graft contouring procedures,[65–69] skin rejuvenation[70] and chronic connective tissue disorders.[71] There have also been three-small

clinical studies that have shown a beneficial effect on wound healing.[40,72,73] Although these clinical benefits have suggested improved patient satisfaction,[74] the scientific evidence for the mechanistic relationship between fat and PRP is limited. In particular, the evidence for the synergistic regenerative relationship is scarce, with the majority of articles focusing on the improved survival and retention of fat grafts. Below, we summarise the evidence from in vitro studies regarding the relationship between fat and PRP with regard to wound healing.

It has been hypothesised that the fibrin component of PRP may act as a scaffold for adipocytes and ADSCs, retaining them at the graft site for longer.[75,76] The fibrin scaffold may also reduce apoptotic cell death in differentiated adipocytes.[77] Siegel et al. showed that ADSCs retained in a fibrin clot show consistently higher secretion of VEGF and FGF as well as enhanced ADSCs immunoreactivity to VEGF suggesting a synergistic effect between the fibrin clot and the stem cells.[78] PRP also contains cell adhesion molecules including fibronectin and vitronectin, which help to immobilise growth factors within the fibrin and help the fibrin scaffold act as a matrix for epithelial migration.[46,76] When ADSCs are grown in vitro on a scaffold containing adhesion molecules and growth factors found in PRP, they show increased differentiation to keratinocytes suggesting an enhanced benefit to wound healing.[79] It has also been shown in vitro that a majority of platelets remain active at 10 days when cocultured with ADSCs indicating a bilateral prosurvival relationship that may enhance healing potential.[80]

PRP has anti-inflammatory properties that may reduce the inflammation and swelling, which encourage degeneration of the fat graft.[81] The increased concentration of the hepatocyte growth factor (HGF) and tumour necrosis factor α (TNFα) in PRP may play a crucial anti-inflammatory role through downregulation of the proinflammatory transcription factor NF-kB, which improves the survival of cocultured cells.[82] ADSCs cocultured with PRP secrete low chemokine concentrations[83] and PRP also encourages downregulation of the strongly proinflammatory gene IL1B in ADSCs both of which suggest an anti-inflammatory effect.[84] ADSCs cultured with PRP from dolphins also show an enhanced ability to phagocytose suggesting a role in the inflammation stage of healing.[85]

Several in vitro studies have shown that PRP enhances proliferation of ADSCs[39,68,86–88] and it can be used as a safe and reliable alternative to standard expansion media.[89,90] This enhanced proliferative effect on ADSCs does not affect their ability to differentiate[91] and a higher volume of ADSCs within a fat graft has been shown to have a positive effect on graft take and survival.[92] One study also found that PRP reduces apoptosis of preadipocytes through the downregulation of the mediator of cell death mRNA proteins and inhibition of proapoptotic genes and this may then enhance the fat survival after transplantation.[93] Li et al. found that in mice PRP upregulated adipogenic gene expression in ADSCs suggesting a beneficial effect on fat growth.[94] However, the concentration of PRP needed for optimum growth of ADSCs is uncertain[45] with several articles quoting approximately 5%-15%[84,95,96] but with high concentrations of 40%-50% PRP leading to cell death[83] because of a negative regulatory effect of platelets on growth factors.[97] Furthermore, some studies have found that, although proliferation increases PRP alone is not sufficient to increase the adipogenesis of the ADSCs.[86,98] Amable et al. also found that PRP did not increase the adipogenic potential of ADSCs or bone marrow-derived stem cells[83] and Chignon-Sicard et al. found that at 20% concentration PRP may actually inhibit adipogenic differentiation.[99]

ADSCs have also been shown to have the potential to differentiate into endothelial cells, which go on to form capillary tubes, a crucial step in angiogenesis.[61,100] in vitro studies illustrate that when PRP and ADSCs are cultured in combination, the growth of vascular networks is increased.[101] A study in mice has also showed that fat grafted in combination with PRP increased neovascularisation of adipose tissue.[102] PRP has been shown to encourage ADSCs to differentiate into fibroblasts and keratinocytes[103] that are crucial cells in the wound-healing process. PRP also encourages migration of fibroblasts to a wound site.[104] ADSCs cultured with PRP express genes such as MMP1 and MMP2 that are involved in tissue remodelling, suggesting a beneficial effect to wound healing.[84]

6 | GROWTH FACTORS AND THEIR ROLE IN PRP AND FAT SYNERGY

Growth factors, including IGF, PDGF, VEGF, TGF, and FGF, found within both fat and PRP may play a role in the wound-healing properties of both treatments. When the treatments are combined, these growth factors may have a synergistic effect on adipogenesis and the survival of transplanted fat.

PDGF is an important growth factor in wound healing in general as it stimulates migration, proliferation, and differentiation of a wide variety of wound-healing cells[105,106] as well as encouraging angiogenesis.[107] Direct topical application of PDGF gel to chronic ulcers has been shown to have a positive effect on wound healing.[108] In vitro, it has been shown that PDGF encourages differentiation of preadipocytes in a manner similar to the differentiation process in serum in vivo.[109] Withdrawal of PDGF from growing adipocytes can lead to apoptosis and reduced differentiation.[110] Animal studies have shown that PDGF signalling via the PDGF beta receptor (PDGFRβ) is crucial in the neovascularisation of adipose tissue.[111] The pharmacological inhibition of this receptor may also reduce proliferation and migration of ADSCs.[112] Long-term delivery of PDGF also increases fat graft weight and architectural survival in mice.[113] PDGF

also increases the angiogenesis potential of ADSCs via increased differentiation to endothelial cells.[60] PDGF may also stimulate the release of extracellular vesicles from ADSCs, which plays a major proangiogenic role via the c-kit-SCF-signalling pathway.[114] PDGF is found in high concentrations in PRP[115] and its release is sustained over a period of several days[116] suggesting that PDGF may have an important role in PRP-enhanced fat graft survival and wound healing.

VEGF plays a key role in wound healing via its effect on angiogenesis and vascular permeability through the stimulation of endothelial cell differentiation and migration.[117] These effects may have an impact on wound healing, with the application of recombinant VEGF to diabetic foot ulcers (DFUs) showing positive wound-healing effects.[118] Adipose tissue is known to be an important source of VEGF[119,120] suggesting one of the reasons why fat grafting may be effective in wound healing. VEGF is also found in high concentrations of PRP[121–123] and is directly related to platelet concentration.[116] VEGF is raised in several different PRP preparation methodologies[116] and is released consistently over several days.[124,125] When PRP and ADSCs are transplanted in mice, the levels of VEGF are significantly raised compared with ADSCs or PRP alone.[126] The synergistic effect on angiogenesis of VEGF from both PRP and fat may be a factor in combined wound-healing benefits, although there is little literature to support this hypothesis. VEGF also has a complex role in adipocyte survival and differentiation but the exact function is not understood.[127]

Transforming growth factor beta 1 (TGF) is involved in all stages of wound healing including angiogenesis, inflammation, cell differentiation, and extracellular matrix deposition.[128,129] Experimental models have shown that there is down regulation of TGF-signalling pathways in chronic non-healing wounds.[130,131] TGF has been shown to have a positive effect on the proliferation of prohealing cells[132] and on the synthesis and deposition of collagen[133] when transplanted with PRP. TGF is found at high levels within PRP and can be released over a sustained period[134] or in a bimodal manner;[124] however, its highest concentrations are seen within the first few hours.[121,125] The amount of TGF released appears to be directly related to the platelet concentration suggesting that higher volumes in PRP cause a greater effect.[135] TGF is also released by adipose tissue and release is enhanced in higher volumes of fat,[136] suggesting that surviving fat grafts would continue to release TGF within wounds to encourage healing. TGF has also been shown in animal models to have a positive effect on adipogenesis[137] therefore suggesting that an increased concentration in PRP may have synergistic effects on both fat survival and wound healing. It has also been shown that PRP stimulates ADSC differentiation into myofibroblasts, crucial cells in the remodelling phase of wound healing, via the TGFβ1-signalling pathway.[99] However, the same study also showed that the antiadipogenic effects caused by higher concentrations of PRP (20%) are controlled through the TGFβ1-signalling pathway.

FGF has proangiogenic functions that are important in wound healing.[138,139] FGF is also involved in the differentiation, migration, and proliferation of a wide variety of wound-healing cells.[140] In particular, the ligand FGF-2 has been shown to have an important role in wound healing and re-epithelialisation[141] with down regulation of FGF-2 in mice shown to have negative effects on wound healing.[142] FGF2 can also stimulate the differentiation of ADSCs to endothelial cells to enhance angiogenesis.[143] FGF is found in increased volumes in PRP[144] and is released rapidly from PRP at high concentrations with maximum levels detected at 1 hour[116] suggesting that it may assist in the early phases of wound healing. One animal study demonstrated a synergistic effect of PDGF and FGF in enhancing angiogenesis and the growth of functional and stable vascular networks in mice.[145] However, the role of FGF in growth and survival of fat cells is complex and not well defined. Some studies have shown that FGF1 is important in the proliferation and differentiation of adipocytes[146] and FGF2 may be involved in the regeneration of ADSCs.[147] However, some studies have shown that FGF is a negative adipogenic factor[148] and that FGF at low concentrations may have the most proadipogenic effect.[149]

IGF is a growth factor needed by a large number of different cells for survival, proliferation, and apoptosis modulation.[150] It is also a key mediator of preadipocyte proliferation, differentiation, and survival[151] and although it is principally released from the liver, it is also secreted from local tissue including fat[152] and is found in high concentration in activated PRP.[121,124] Animal models have shown that topical application of IGF1 can increase re-epithelialisation of wounds.[153] The delivery of IGF in combination with insulin and FGF has been shown to increase fat graft survival.[154] When cocultured, PRP enhances proliferation of ADSCs via the IGF-1-signalling pathway.[155]

7 | PRP AND FAT IN CHRONIC DIABETIC WOUNDS

The role of PRP in improving fat survival may also have important consequences for wound healing in patients with diabetic wounds. Diabetes is one of the world's most common diseases and a large minority of patients will go on to develop non-healing wounds.[156] Conventional treatment options of wound care and infection prevention are costly to the health service with inconsistent efficacy.[157] Diabetic wounds are in a state of chronic inflammation with impairment in all the physiological and biochemical wound-healing processes including angiogeneis, cellular differentiation, and growth factor production.[158] Given the prevalence of diabetic wounds, the regenerative potential of fat, the low cost,

safe, and straightforwardness of conventional fat grafting, there may be a role for it in treating these patients. Some animal studies have shown an improvement in re-epithlialisation and granulation when diabetic wounds were treated with ADSCs.[159–161] One clinical study has also shown positive results with standard Coleman fat grafting to chronic diabetic wounds.[14]

However, the benefits of fat grafting, and in particular ADSCs, in diabetes have been questioned with authors suggesting the systemic effects of the disease reduce the regenerative capacity of ADSCs[162] One concern is that the volume of ADSCs found in the SVF of diabetic patients is reduced[163] and the stem cells are more prone to cell death with reduced capacity for differentiation.[164] Diabetic ADSCs may also secrete fewer growth factors that may impair their healing function.[165] In the rat model, diabetes significantly reduces the viability of ADSCs through increased apoptosis and down regulation of genes needed for stem cell maintenance and growth.[166] In the same study, the authors also illustrated a significantly reduced capacity of diabetic ADSCs to form capillary networks. However, the authors also found that the potential for ADSCs to differentiate into adipocytes was not inhibited. In another rat model, the authors found reduced proliferation of diabetic ADSCs but also a significantly reduced proangiogenic function.[167]

The addition of PRP to diabetic fat may help reverse some of the negative effects on ADSCs. The role of PRP and its growth factors alone has been shown to have positive diabetic wound-healing results in the literature. Direct infusion of PRP to the wound bed[168] and topical application of PDGF have both shown positive results in DFU healing.[169] VEGF, whose receptor may be downregulated in DFU,[170] has shown a shorter time to healing of DFUs when applied topically.[171,172] IGF has been found to be downregulated in diabetic fibroblasts[173] suggesting that the PRP may have a beneficial effect on IGF function in diabetics. However, evidence for combined treatment in both preclinical and clinical studies is limited in diabetes and further work is necessary. The authors have embarked on a randomised controlled clinical trial to evaluate the effect of both fat and fat with PRP on wound healing in DFUs to evaluate the above hypotheses and add to the body of evidence.

8 | PRP AND FAT IN ANIMAL STUDIES

In vitro studies tend to focus on individual factors are unlikely to represent the true relationship of fat and PRP and most of the theory is interpreted to be relevant to wound healing rather than being directly applicable. In reality, the regenerative relationship between PRP and fat is likely to be highly complex involving a network of growth factors, cells, cytokines, and other prohealing molecules, which interact continuously in both space and time, a scenario that is impossible to recreate in the laboratory. Therefore, further robust animal and clinical studies are necessary to understand the true relationship and how this translates to clinical benefit. Below, we summarise the available evidence from animal studies on the effect of fat and PRP in wound healing.

The ease of comparison of animal studies, as with clinical studies, is made difficult by the wide variety in methodology including differences in PRP preparation technique and source, fat harvest and preparation methods, constitution of PRP, and different graft sites. Despite this, there have been several animal studies that have shown a benefit to graft retention in contouring procedures with PRP being shown to enhance the vascularity and reduce fat necrosis of transplanted fat.[174–177] In a rat model, one study found that activated PRP enhances fat graft retention as well as the survival, viability, and differentiation of adipocytes.[178] Pires Fraga et al. also found that PRP not only improved graft retention but also maintained a higher number of adipocytes and blood vessels with reduced necrosis and fibrosis in a rabbit model.[179] In another rabbit model, Rodriguez-Flores et al. found that combination fat and PRP grafting reduced the inflammatory reaction and the accumulation of oil cysts (indicating fat necrosis) within adipocytes.[180] However, Kim et al. found that at 12 weeks the volume of fat graft retention when transplanted with PRP was not significantly different compared with fat alone.[181] All of these studies analysed the effect of PRP on fat graft survival in animals rather than any regenerative function; however, their findings can be applicable to the potential benefit for wound healing given what we discussed above.

Animal studies evaluating the effect of fat and PRP on wound healing are extremely limited in the literature with only one published study. Blanton et al. used a porcine model to evaluate the effect of ADSCs and PRP on the healing of full thickness wounds.[182] In this study, the authors processed fat to isolate and culture ADSCs for 1 week prior to grafting. The ADSCs were then mixed with either platelet-poor plasma (PPP) or PRP, they also included PPP, PRP, and saline controls. Each wound was filled with the treatment mix and followed up for 3 weeks, there was no injection of the fat into the wound edge or base as would be expected practice in a clinical scenario. They found no improvement in reepithelialisation of wounds with any treatment but there was a significant increase in vascularity of wounds treated with ADSC/PRP mix with a corresponding significant increase in secretion of VEGF. They also found a significant improvement in the cosmesis of wounds with the ADSC/PRP mix when using a subjective wound evaluation score.

9 | CONCLUSION AND FUTURE CONSIDERATIONS

In conclusion, there is a relatively broad body of evidence, in both animals and humans, to suggest that PRP enhances

the survival of fat. This probably occurs through an improvement in the vascularisation of transplanted fat and the formation of a fibrin scaffold, which retains essential cells and growth factors within the graft. There may also be an anti-inflammatory role of PRP, which prevents fat necrosis and cell death. However, the conclusions of several studies are limited by heterogenous methodology and small sample sizes, with a lack of randomised controlled clinical trials. Furthermore, there is limited evidence to suggest a beneficial effect on wound healing of combining fat and PRP, with only one animal and three small human studies in the literature. However, preclinical studies have illustrated that the theory behind a synergistic regenerative effect of PRP on fat is logical. PRP provides a clear proliferative benefit to the expansion of ADSCs, and it encourages the formation of vascular networks when combined with ADSCs, a finding that has translated into animal studies. The growth factors found within both fat and PRP at high concentrations have shown in preclinical, animal, and human studies to have a beneficial effect on wound healing and several studies have found that these factors also enhance fat survival.

However, there are significant question marks over the clinical applicability of ADSCs because of cost, logistics, and governmental restriction. The majority of preclinical and animal studies focus specifically on the regenerative potential of ADSCs; however, routine fat grafting is cheap, safe, simple, and is widely used in clinical practice. Given the restrictions on ADSCs, further studies should focus on investigating fat grafts and methods to improve their regenerative capabilities that are sensible in the current financial climate.

Further considerations for future studies are to define the concentration of PRP needed to give an optimum effect to fat, with some studies illustrating a deleterious effect of high concentrations of both PRP and certain growth factors. Further studies must also attempt to develop a clear and reproducible methodology for both fat and PRP harvest and mixing that gives the optimum regenerative effect. Given the ease of harvest and very low risk in harvesting both PRP and fat, the clinical use of both will continue despite the lack of evidence. Therefore, robustly conducted randomised controlled clinical trials to evaluate the effect on wound healing are essential. Studies to better understand the mechanistic relationship between both fat and PRP and how this translates to wound healing are also essential to better guide further research and clinical practice.

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