Abstract

Fat grafting is becoming a common procedure in regenerative medicine due to 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 have the potential to differentiate into a wide range of wound healing cells including fibroblasts and keratinocytes as well as demonstrating pro-angiogenic qualities. This suggests 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 due to 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.

Introduction

Autologous fat grafting is a popular procedure in plastic and reconstructive surgery due to 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 fat extracellular matrix, and secreted from

the ADSCs themselves, are pro-healing growth factors, anti-inflammatory cytokines, pro-angiogenic 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 careful and meticulous technique or appropriate patient selection. However, inadequate neo-vascularisation 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 in order to improve its retention rate through the improvement of neovascularisation, including stromal vascular fraction (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 due to wide variation in PRP preparation methodology leading to an unreliable and non-reproducible growth factor content (38).

PRP contains an abundance of growth factors, pro-angiogenic 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 pro-healing 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).

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 platelet rich plasma 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).

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 which 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 which adheres to damaged endothelial surfaces of blood vessels. Strands of fibrin reinforce the plug forming a thrombus which 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 in order 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 pro-healing cells. The fibrin matrix of the haemostatic clot is replaced with granulation tissue by fibroblasts with the deposition of type 3 collagen and extracellular matrix which provides a nutrient supply and scaffold for angiogenesis. Remodelling and maturation of the granulation tissue then occurs 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 (e.g. infection, ischaemia, foreign bodies) and systemic factors (e.g. age, immunosuppression, diabetes). Enhancing the factors which 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.

Platelet rich plasma

Platelet rich plasma is obtained via the centrifugation of whole blood which is obtained via peripheral venipuncture and mixed with acid-citrate dextrose (ACDA). There are a wide range of commercially available kits which 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 which is often contradictory or difficult to compare.

Platelet rich plasma 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 micro-litre is thought to be clinical beneficial (46,47). Alpha granules contained within platelets contain many growth factors which are known to be pro-healing 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 which are involved in cell migration and growth factor release, and pro-inflammatory molecules such as serotonin and histamine which encourage the inflammation stage of wound healing (30).

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, aqueous solution) (49). The lipoaspirate is the tissue that is grafted and contains two principle components which are not separated before conventional grafting: mature adipocytes and stromal vascular fraction (SVF). Further centrifugation of the lipoaspirate would produce a cell-dense pellet within the lower layer which would contain the SVF and ADSCs which 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 (50-54), the clinical application of ADSCs is extremely limited in clinical practice due to cost, logistical issues and ethical concerns (55) with governmental restrictions in place in many countries including the USA.

The SVF of the fat graft contains pre-adipocytes and multipotent ADSCs which have the potential to differentiate into wound healing cells such as fibroblasts, endothelial cells and keratinocytes (6, 56). ADSCs have been shown to encourage neo-vascularisation and tissue regeneration in vivo (57, 58). Adipose tissue has one of the highest concentrations of stem cells in the body, with approximately 5000 ADSCs per gram of fat (59). With regard to wound healing, the ADSCs may contribute anti-inflammatory and pro-angiogenic function through the paracrine secretion of soluble mediators (60). 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 (61). 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 (62, 63).

However the main limitation of fat grafting is inconsistent survival of the graft (64) 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 (65) 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 (66). It is reasonable to hypothesise that a fat graft that survives for longer would have a improved healing outcome as it would continue to release pro-healing mediators as well as allowing ADSCs to differentiate for longer.

The role of PRP in fat graft survival

There have been several clinical studies which have shown a beneficial effect of PRP on the outcome of fat graft contouring procedures (67-71), skin rejuvenation (72) and chronic connective tissue disorders (73). There have also been three small clinical studies which have shown a beneficial effect on wound healing (40, 74-75). However, although these clinical benefits have suggested improved patient satisfaction (76), 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 papers 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 regards 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 (77, 78). The fibrin scaffold may also reduce apoptotic cell death in differentiated adipocytes (79). 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 (80). 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 (46,78). 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 (81). It has also been shown in vitro that a majority of platelets remain active at 10 days when co-cultured with ADSCs indicating a bilateral pro-survival relationship which may enhance healing potential (82).

PRP has anti-inflammatory properties which may reduce the inflammation and swelling which encourage degeneration of the fat graft (83). The increased concentration of hepatocyte growth factor (HGF) and tumour necrosis factor α (TNFα) in PRP may play a crucial anti-inflammatory role through down regulation of the pro-inflammatory transcription factor NF-kB which improves the survival of co-cultured cells (84). ADSCs co-cultured with PRP secrete low chemokine concentrations (85) and PRP also encourages down regulation of the strongly pro-inflammatory gene IL1B in ADSCs both of which suggest an anti-inflammatory effect (86). ADSCs cultured with PRP from dolphins also show an enhanced ability to phagocytose suggesting a role in the inflammation stage of healing (87).

Several in vitro studies have shown that PRP enhances proliferation of ADSCs (39, 70, 88-90) and it can be used as a safe and reliable alternative to standard expansion media (91,92). This

enhanced proliferative effect on ADSCs does not affect their ability to differentiate (93) and a higher volume of ADSCs within a fat graft has been shown to have a positive effect on graft take and survival (94). One study also found that PRP reduces apoptosis of preadipocytes through the down regulation of mediator of cell death mRNA proteins and inhibition of pro-apoptotic genes and this may then enhance the fat survival after transplantation (95). Li et al found that in mice PRP upregulated adipogenic gene expression in ADSCs suggesting a beneficial effect on fat growth (96). However the concentration of PRP needed for optimum growth of ADSCs is uncertain (45) with several papers quoting approximately 5-15% (86, 97, 98) but with high concentrations of 40-50% PRP leading to cell death (85) due to a negative regulatory effect of platelets on growth factors (99). Furthermore, some studies have found that, although proliferation increases PRP alone is not sufficient to increase the adipogenesis of the ADSCs (88, 100). Amable et al also found that PRP did not increase the adipogenic potential of ADSCs or bone marrow derived stem cells (85) and Chignon-Sicard et al found that at 20% concentration PRP may actually inhibit adipogenic differentiation (101).

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 (63, 102). In vitro studies illustrate that when PRP and ADSCs are cultured in combination the growth of vascular networks is increased (103). A study in mice has also showed that fat grafted in combination with PRP increased neovascularisation of adipose tissue (104). PRP has been shown to encourage ADSCs to differentiate into fibroblasts and keratinocytes (105) which are crucial cells in the wound healing process. PRP also encourages migration of fibroblasts to a wound site (106). ADSCs cultured with PRP express genes such as MMP1 and MMP2 which are involved in tissue remodelling, suggesting a beneficial effect to wound healing (86).

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 (107, 108) as well as encouraging angiogenesis (109). Direct topical application of PDGF gel to chronic ulcers has been shown to have a positive effect on wound healing (110). In vitro it has been shown that PDGF encourages differentiation of preadipocytes in a manner similar to the differentiation process in serum in vivo (111). Withdrawal of PDGF from growing adipocytes can lead to apoptosis and reduced differentiation (112). Animal studies have shown that PDGF signalling via the PDGF beta receptor (PDGFRβ) is crucial in the neovascularisation of adipose tissue (113). The pharmacological inhibition of this receptor may also reduce proliferation and migration of ADSCs (114). Long term delivery of PDGF also increases fat graft weight and architectural survival in mice (115). PDGF also increases the angiogenesis potential of ADSCs via increased differentiation to endothelial cells (62). PDGF may also stimulate the release of extracellular vesicles from ADSCs which play a major pro-angiogenic role via the c-kit-SCF signalling pathway (116). PDGF is found in high concentrations in PRP (117) and its release is sustained over a period of several days (118) suggesting that PDGF may have a 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 (119). These effects may have an impact on wound healing, with the application of recombinant VEGF to diabetic foot ulcers showing positive wound healing effects (120). Adipose tissue is known to be an important source of VEGF (121,122) suggesting one of the reasons why fat grafting may be effective in wound healing. VEGF is also found in high concentrations of PRP (123-5) and is directly related to platelet concentration (118). VEGF is raised in several different PRP preparation methodologies (118) and is released consistently over several days (126, 127). When PRP and ADSCs are transplanted in mice the levels of VEGF are significantly raised compared to ADSCs or PRP alone (128). 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 (129).

Transforming growth factor beta 1 (TGF) is involved in all stages of wound healing including angiogenesis, inflammation, cell differentiation and extracellular matrix deposition (130, 131). Experimental models have shown that there is down regulation of TGF signalling pathways in chronic non-healing wounds (132,133). TGF has been shown to have a positive effect on the proliferation of pro-healing cells (134) and on the synthesis and deposition of collagen (135) when transplanted with PRP. TGF is found at high levels within PRP and can be released over a sustained period (136) or in a bimodal manner (126), however its highest concentrations are seen within the first few hours (123, 127). The amount of TGF released appears to be directly related to the platelet concentration suggesting higher volumes in PRP cause a greater effect (137). TGF is also released by adipose tissue and release is enhanced in higher volumes of fat (138), 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 (139) 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 (101). However the same study also showed that the anti-adipogenic effects caused by higher concentrations of PRP (20%) are controlled through the TGFβ1 signalling pathway.

Fibroblast growth factor (FGF) has pro-angiogenic functions which are important in wound healing (140, 141). FGF is also involved in the differentiation, migration and proliferation of a wide variety of wound healing cells (142). In particular the ligand FGF-2 has been shown to have an important role in wound healing and re-epithelialisation (143) with down regulation of FGF-2 in mice shown to have negative effects on wound healing (144). FGF2 can also stimulate the differentiation of ADSCs to endothelial cells to enhance angiogenesis (145). FGF is found in increased volumes in

PRP (146) and is released rapidly from PRP at high concentrations with maximum levels detected at one hour (118) suggesting 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 (147). However the role of FGF in growth and survival of fat cells is complex and not well defined. Some studies have shown FGF1 is important in the proliferation and differentiation of adipocytes (148) and FGF2 may be involved in the regeneration of ADSCs (149). However some studies have shown that FGF is a negative adipogenic factor (150) and that FGF at low concentrations may have the most pro-adipogenic effect (151).

IGF is a growth factor needed by a large number of different cells for survival, proliferation and apoptosis modulation (152). It is also a key mediator of preadipocyte proliferation, differentiation and survival (153) and, although it is principally released from the liver, it is also secreted from local tissue including fat (154) and is found in high concentration in activated PRP (123,126). Animal models have shown topical application of IGF1 can increase re-epithelialisation of wounds (155). The delivery of IGF in combination with insulin and FGF has been shown to increase fat graft survival (156). When co-cultured, PRP enhances proliferation of ADSCs via the IGF-1 signalling pathway (157).

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 worlds most common diseases and a large minority of patients will go on to develop non-healing wounds (158). Conventional treatment options of wound care and infection prevention are costly to the health service with inconsistent efficacy (159). 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 (160). Given the prevalence of diabetic wounds, the regenerative potential of fat, and 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 (161-163). 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 has been questioned with authors suggesting the systemic effects of the disease reduces the regenerative capacity of ADSCs (164). One concern is that the volume of ADSCs found in the SVF of diabetic patients is reduced (165) and the stem cells are more prone to cell death with reduced capacity for differentiation (166). Diabetic ADSCs may also secrete fewer growth factors which may impair their healing function (167). 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 (168). 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 (169).

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 have been shown to have positive diabetic wound healing results in the literature. Direct infusion of PRP to the wound bed (170) and topical application of PDGF have both shown positive results in diabetic foot ulcer (DFU) healing (171). VEGF, who's receptor may be down regulated in DFU (172), has shown shorter time to healing of DFUs when applied topically (173, 174). IGF has been found to be down regulated in diabetic fibroblasts (175) suggesting that the PRP may have a beneficial effect on IGF function in diabetics. However evidence for combination 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 in order to evaluate the above hypotheses and add to the body of evidence.

PRP and fat in animal studies

In vitro studies tend to focus on individual factors is unlikely to represent the true relationship of fat and PRP and a lot 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 highly complex involving a network of growth factors, cells, cytokines and other pro-healing 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 which 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 (176-179). In a rat model, one study found that activated PRP enhances fat graft retention as well as the survival, viability and differentiation of adipocytes (180). 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 (181). 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 (182). However Kim et al found that at 12 weeks the volume of fat graft retention when transplanted with PRP was not significantly different compared to fat alone (183). 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 (184). In this study the authors

processed fat in order to isolate and culture ADSCs for one 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 three 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 re-epithelialisation 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.

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 samples 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 surivival.

However, there are significant question marks over the clinical applicability of ADSCs due to cost, logistics, and governmental restriction. The majority of pre-clinical 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 its 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 in order to better guide further research and clinical practice.

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References

1. Coleman SR. Structural fat grafting: More than a permanent filler. Plast Reconstr Surg. 2006;118(Suppl):108S–120S.
2. Fredman R, Katz AJ, Hultman CS. Fat grafting for burn, traumatic and surgical scars. Clin Plast Surg. 2017;44(4):781-91
3. Griffin MF, Almadori A, Butler PE. Use of lipotransfer in scleroderma. Aesthet Surg J. 2017;37:S33-37
4. Pu LLQ, Yoshimura K, Coleman SR. Fat grafting: current concept, clinical application, and regenerative potential, part 1. Clin Plast Surg. 2015;42(2):ix–x.
5. Pu LL, Yoshimura K, Coleman SR. Fat grafting: current concept, clinical application, and regenerative potential, part 2. Preface. Clin Plast Surg. 2015;42:xiii–xxiv.
6. Zuk PA, Zhu M, Mizuno H, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng. 2001;7:211–228
7. Fromm-Dornieden C, Koenen P. Adipose-derived stem cells in wound healing: Recent results in vitro and in vivo. OA Mol Cell Biol. 2013;1:8
8. Li PB, Jin H, Liu DX, et al. Study on leptin enhancing collagen synthesis in wounded rats. Zhongguo Ying Yong Sheng Li Xue Za Zhi 2011;27:72–74.
9. Piccolo, N. S., Piccolo, M. S. and Piccolo, M. T. S. (2015) 'Fat Grafting for Treatment of Burns, Burn Scars, and Other Difficult Wounds', *Clinics in Plastic Surgery*. Elsevier, 42(2), pp. 263–283
10. Herold C, Rennekampff HO, Groddeck R, Allert S. Autologous fat transfer for thumb carpometacarpal joint osteoarthritis: a prospective study. Plast Reconstr Surg. 2017;140(2):327-35
11. Rigotti G, Marchi A, Gali. M, et al. Clinical treatment of radiotherapy tissue damage by lipoaspirate transplant: A healing process mediated by adipose-derived adult stem cells. Plast Reconstr Surg. 2007;119:1409–1422; discussion 1423.
12. Marino G, Moraci M, Armenia E et al. Therapy with autologous adipose-derived regenerative cells for the care of chronic ulcer of lower limbs in patients with peripheral arterial disease. J Surg Res. 2013; 185: 36-44

13. Marangi GF, Pallara T, Cagli B, et al. Treatment of early-stage pressure ulcers by using autologous adipose tissue grafts. Plast Surg Int. 2014;2014:817283.
14. Stasch T et al. Debridement and Autologous Lipotransfer for Chronic Ulceration of the Diabetic Foot and Lower Limb Improves Wound Healing. Plast Reconstr Surg. 2015;136(6):1357-66
15. Rehman J, Traktuev D, Li J, et al. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. Circulation. 2004;109:1292–1298.
16. Sinna R, Delay E, Garson S, et al. Breast fat grafting (lipomodelling) after extended latissimus dorsi flap breast reconstruction: a preliminary report of 200 consecutive cases. J Plast Reconstr Aesthet Surg. 2010;63:1769–1777.
17. Missana MC, Laurent I, Barreau L, et al. Autologous fat transfer in reconstructive breast surgery: indications, technique and results. Eur J Surg Oncol. 2007;33:685–690.
18. Delay E, Garson S, Tousson G, et al. Fat injection to the breast: technique, results, and indications based on 880 procedures over 10 years. Aesthet Surg J. 2009;29:360–376.
19. Conde-Green A, de Amorim NF, Pitanguy I. Influence of decantation, washing and centrifugation on adipocyte and mesenchymal stem cell content of aspirated adipose tissue: a comparative study. J Plast Reconstr Aesthet Surg. 2010;63:1375–1381.
20. Zhu M, Cohen SR, Hicok KC, Shanahan RK, Strem BM, Yu JC, Arm DM, Fraser JK. Comparison of three different fat graft preparation methods: gravity separation, centrifugation, and simultaneous washing with filtration in a closed system. Plast Reconstr Surg. 2013;131(4):873-80
21. Varghese J, Griffin M, Mosahebi A, et al. Systematic review of patient factors affecting adipose stem cell viability and function: implications for regenerative therapy. Stem Cell Res Ther. 2017;8:45.
22. Bartynski J,Marion MS,Wang TD. Histopathologic evaluation of adipose autografts in a rabbit ear model. Otolaryngol Head Neck Surg 1990;102:314–21.
23. Crandall DL, Hausman GJ, Kral JG. A review of the microcirculation of adipose tissue: anatomic, metabolic, and angiogenic perspectives. Microcirculation 1997;4:211–232.

24. Sommer B, Sattler G. Current concepts of fat graft survival: histology of aspirated adipose tissue and review of the literature. Dermatol Surg 2000;26:1159–66.
25. Carpaneda CA, Ribeiro MT. Percentage of graft viability versus injected volume in adipose autotransplants. Aesthetic Plast Surg. 1994;18:17–19.
26. Baran CN, Celebioğlu S, Sens.z O, et al. The behavior of fat grafts in recipient areas with enhanced vascularity. Plast Reconstr Surg. 2002;109:1646–1651; 1652.
27. Lee SK, Kim DW, Dhong ES, Park SH, Yoon ES. Facial Soft Tissue Augmentation using Autologous Fat Mixed with Stromal Vascular Fraction. Arch Plast Surg. 2012;39(5):534-9
28. Yu P, Zhai Z, Jin X, Yang X, Qi Z. Clincal application of platelet rich fibrin in plastic and reconstructive surgery: a systematic review. Aesthet Plast Surg. 2018;42(2):511-19
29. Frechette JP, Martineau I, Gagnon G. Platelet-rich plasmas: growth factor content and roles in wound healing. J Dent Res. 2005;84(5):434-9
30. Foster TE, Puskas BL, Mandelbaum BR, et al. Platelet-rich plasma: from basic science to clinical applications. Am J Sports Med. 2009;37:2259–2272.
31. Sommeling CE, Heyneman A, Hoeksema H, et al. The use of platelet-rich plasma in plastic surgery: a systematic review. J Plast Reconstr Aesthet Surg. 2013;66:301–311.
32. Venter NG, Marques RG, Santos JS, Monte-Alto-Costa A. Use of platelet rich plasma in deep second and third degree burns. Burns. 2016;42(4):807-14
33. Cervantes J, Perper M, Wong LL, Eber AE, Villasante Fricke AC, Wikramanayake TC, Jimenez JJ. Effectiveness of platelet rich plasma for androgenetic alopecia: a review of the literature. Skin Appendage Disord. 2018;4(1):1-11
34. Ye Y, Zhou X, Mao S, Zhang J, Lin B. Platelet rich plasma versus hyaluronic acid in patients with hip osteoarthritis: A meta-analysis of randomized controlled trials. Int J Surg. 2018;53:279-87
35. Hurley ET, Lim Fat D, Moran CJ, Mullett H. The Efficacy of Platelet-Rich Plasma and Platelet-Rich Fibrin in Arthroscopic Rotator Cuff Repair: A Meta-analysis of Randomized Controlled Trials. Am J Sports Med. 2018;1 (Epub ahead of print)

36. Ikumi A, Hara Y, Yoshioka T, Kanamori A, Yamazaki M. Effect of local administration of platelet-rich plasma (PRP) on peripheral nerve regeneration: An experimental study in the rabbit model. Microsurgery. 2018;38(3):300-9
37. Martinez-Zapata MJ, Mart.-Carvajal AJ, Sol. I, et al. Autologous platelet-rich plasma for treating chronic wounds. Cochrane Database Syst Rev. 2016;2016:CD006899
38. Leitner GC, Gruber R, Neumu¨ller J, Wagner A, Kloimstein P, Hocker P, Kormoczi GF, Buchta C. Platelet content and growth factor release in platelet-rich plasma: a comparison of four different systems. Vox Sang.2006;91:135–139
39. Kakudo N, Minakata T, Mitsui T et al. Proliferation-promoting effect of platelet-rich plasma on human adipose-derived stem cells and human dermal fibroblasts. Plast Reconstr Surg. 2008;122(5):1352-60
40. Raposio E, Bertozzi N, Bonomini S, Bernuzzi G, Formentini A, Grifnaffini E, Pio Grieco M. Adipose derived stem cell added to platelet rich plasma for chronic skin ulcer therapy. Wounds. 2016;28(4):126-31
41. Smith OJ, Kanapathy M, Khajuria A, Prokopenko M, Hachach-Haram N, Mann H, Mosahebi A. Systematic review of the efficacy of fat grafting and platelet rich plasma for wound healing. Int Wound J. 2018. Epub ahead of print.
42. Luck J, Smith OJ, Mosahebi A. A systematic review of autologous platelet rich plasma and fat graft preparation methods. Plast Recontr Surg Glob Open. 2017.5:e1596.
43. Rittie L. Cellular mechanisms of skin repair in humans and other mammals. J Cell Commun Signal. 2016;10(2):103–120.
44. Wu B, Mottola G, Schaller M, Upchurch GR Jr., Conte MS. Resolution of vascular injury: specialized lipid mediators and their evolving therapeutic implications. Mol Aspects Med. 2017;58:72–82.
45. Liao HT, Marra KG, Rubin JP. Application of platelet-rich plasma and platelet-rich fibrin in fat grafting: basic science and literature review. Tissue Eng Part B Rev. 2014 Aug;20(4):267-76
46. Marx, R.E. Platelet-rich plasma: evidence to support its use. J Oral Maxillofac Surg. 2004;62:489.

47. Eppley, B.L., Pietrzak, W.S., and Blanton, M. Platelet-rich plasma: a review of biology and applications in plastic surgery. Plast Reconstr Surg. 2006;118:147e
48. Pallua N, Pulsfort AK, Suschek C, et al. Content of the growth factors bFGF, IGF-1, VEGF, and PDGF-BB in freshly harvested lipoaspirate after centrifugation and incubation. Plast Reconstr Surg. 2009;123:826–833.
49. Coleman SR. Structural fat grafting: More than a permanent filler. Plast Reconstr Surg. 2006;118(Suppl):108S–120S.
50. Altman AM, Yan Y, Matthias N, Bai X, Rios C, Mathur AB, Song YH, Alt EU. IFATS collection: human adipose-derived stem cells seeded on a silk fibroin-chitosan scaffold enhance wound repair in a murine soft tissue injury model. Stem Cells 2009;27:250–8.
51. Kim W-S, Park B-S, Sung J-H, Yang J-M, Park S-B, Kwak S-J, Park JS. Wound healing effect of adipose-derived stem cells: a critical role of secretory factors on human dermal fibroblasts. J Dermatol Sci. 2007;48:15–24.
52. Liu S, Zhang H, Zhang X, LuW, Huang X, Xie H, Zhou J,Wang W, Zhang Y, Liu Y, Deng Z, Jin Y. Synergistic angiogenesis promoting effects of extracellular matrix scaffolds and adipose-derived stem cells during wound repair. Tissue Eng Part A 2010;17:725–39.
53. Ferraro GA, De Francesco F, Nicoletti G, Paino F, Desiderio V, Tirino V, D'Andrea F. Human adipose CD34+ CD90+ stem cells and collagen scaffold constructs grafted in vivo fabricate loose connective and adipose tissues. J Cell Biochem 2013;114:1039–49.
54. Lu W, Yu J, Zhang Y, Ji K, Zhou Y, Li Y, Deng Z, Jin Y. Mixture of fibroblasts and adipose tissue-derived stem cells can improve epidermal morphogenesis of tissue-engineered skin. Cells Tissues Organs 2012;195:197–206.
55. Naderi N, Combellack EJ, Griffin M et al. The regenerative role of adipose-derived stem cells (ADSC) in plastic and reconstructive surgery. Int Wound J. 2017;14(1):112-124
56. Zhu Y, Liu T, Song K et al. Adipose derived stem cell: a better stem cell than BMSC. Cell Biochem Funct. 2018;26:664–675.
57. Cao Y, Sun Z, Liao L et al. Human adipose tissue-derived stem cells differentiate into endothelial cells in vitro and improve postnatal neovascularization in vivo. Biochem Biophys Res Commun. 2005;332:370–379.

58. Ferraro GA, De Francesco F, Nicoletti G et al. Human adipose CD34+ CD90+ stem cells and collagen scaffold constructs grafted in vivo fabricate loose connective and adipose tissues. J Cell Biochem. 2013;114:1039–1049.
59. Bellini, E., Grieco, M. P. and Raposio, E. The science behind autologous fat grafting. *Annals of Medicine and Surgery*. 2017;24:65–73
60. Prockop DJ, Brenner M, Fibbe WE, Horwitz E, Le BK, Phinney DG, et al. Defining the risks of mesenchymal stromal cell therapy. Cytotherapy. 2010;12:576–8.
61. Trojahn Kølle, S.-F. *et al.* Importance of mesenchymal stem cells in autologous fat grafting: A systematic review of existing studies. *Journal of Plastic Surgery and Hand Surgery. 2012;*46(2):59–68
62. Keerl S, Gehmert S, Gehmert S et al. PDGF and bFGF modulate tube formation in adipose tissue-derived stem cells. Ann Plast Surg. 2010 Apr;64(4):487-90
63. Planat-Benard V, Silvestre JS, Cousin B, et al. Plasticity of human adipose lineage cells toward endothelial cells: physiological and therapeutic perspectives. Circulation. 2004;109:656–663.
64. Nishimura T, Hashimoto H, Nakanishi I, Furukawa M. Microvascular angiogenesis and apoptosis in the survival of free fat grafts. Laryngoscope. 2000;110:1333–8.
65. Eto, H., Kato, H., Suga, H., Aoi, N., Doi, K., Kuno, S.,Yoshimura, K. The fate of adipocytes after nonvascularized fat grafting: evidence of early death and replacement of adipocytes. Plast Reconstr Surg 129, 1081, 2012.
66. Ullmann, Y., Hyams, M., Ramon, Y., Beach, D., Peled, I.J., Lindenbaum, E.S. Enhancing the survival of aspirated human fat injected into nude mice. Plast Reconstr Surg 101,1940, 1998.
67. Chandarana S, Fung K, Franklin JH, Kotylak T, Matic DB and Yoo J. Effect of autologous platelet adhesives on dermal fat graft resorption following reconstruction of a superficial parotidectomy defect: a double-blinded prospective trial. Head Neck 2009; 31: 521-530.
68. Picard F, Hersant B, La Padula S, Meningaud JP. Platelet-rich plasma enriched autologous fat graft in regenerative aesthetic facial surgery: Technical note. J Stomatol Oral Maxillofac Surg 118 (2017) 228–231

69. Jin R, Zhang L, Zhang YG. Does platelet rich plasma enhance the survival of grafted fat? An update review. Int J Clin Exp Med 2013;6(4):252-258
70. Gentile P, Orlandi A, Scioli MG et al. Concise Review: Adipose-Derived Stromal Vascular Fraction Cells and Platelet-Rich Plasma: Basic and Clinical Implications for Tissue Engineering Therapies in Regenerative Surgery. STEM CELLS TRANSLATIONALMEDICINE 2012;1:230–236
71. Gentile P, De Angelis B, Pasin M et al. Adipose-Derived Stromal Vascular Fraction Cells and Platelet-Rich Plasma: Basic and Clinical Evaluation for Cell-Based Therapies in Patients With Scars on the Face. J Craniofac Surg 2014;25: 267-272)
72. Wei H, Gu SX, Liang YD et al. Nanofat-derived stem cells with platelet-rich fibrin improve facial contour remodeling and skin rejuvenation after autologous structural fat transplantation. Oncotarget, 2017;8 (40): 68542-68556
73. Virzi F, Bianca P, Giammona A et al. Combined platelet-rich plasma and lipofilling treatment provides great improvement in facial skin-induced lesion regeneration for scleroderma patients. Stem Cell Research & Therapy (2017) 8:236
74. Cervelli V, Gentile P, Grimaldi M. Regenerative Surgery: Use of Fat Grafting Combined with Platelet-Rich Plasma for Chronic Lower-Extremity Ulcers. Aesthet Plast Surg. 2009;33(3):340-5
75. Cervelli V, De Angelis B, Lucarini L, Spallone D, Balzani A, Palla L, et al. Tissue regeneration in loss of substance on the lower limbs through use of platelet-rich plasma, stem cells from adipose tissue, and hyaluronic acid. Adv Skin Wound Care. 2010;23(6):262–72.
76. Willemsen JCN, Lindenblatt N, Stevens HPJD. Results and long-term patient satisfaction after gluteal augmentation with platelet-rich plasma-enriched autologous fat. Eur J Plast Surg (2013) 36:777–782
77. Anitua E, Sanchez M, Nurden AT, Nurden P, Orive G, Andia I. New insights into and novel applications for platelet-rich fibrin therapies. Trends Biotechnol 2006;24:227-34
78. Kang YH, Jeon SH, Park JY, Chung JH, Choung YH, Choung HW, Kim ES, Choung PH. Platelet-rich fibrin is a Bioscaffold and reservoir of growth factors for tissue regeneration. Tissue Eng Part A 2011;17:349-59

79. Aoyagi Y, Kuroda M, Asada S, Tanaka S, Konno S, Tanio M, Aso M, Okamoto Y, Nakayama T, Saito Y, Bujo H. Fibrin grue is a candidate scaffold for long-term therapeutic protein expression in spontaneously differentiated adipocytes in vitro. Exp Cell Res 2012;318:8-15
80. Siegel KR, Clevenger TN, Clegg DO et al. Adipose Stem Cells Incorporated in Fibrin Clot Modulate Expression of Growth Factors. Arthroscopy: The Journal of Arthroscopic and Related Surgery. 2018;Vol 34(2): pp 581-591
81. Sivan U, Jayakumar K, Krishnan LK. Constitution of Fibrin-Based Niche for In Vitro Differentiation of Adipose-Derived Mesenchymal Stem Cells to Keratinocytes. Biores Open Access. 2014 Dec 1;3(6):339-47
82. Atashi F, Jaconi MEE, Pittet-Cuenod B et al. Autologous Platelet-Rich Plasma: A Biological Supplement to Enhance Adipose-Derived Mesenchymal Stem Cell Expansion. Tissue Eng Part C Methods. 2015 Mar;21(3):253-62
83. El-Sharkawy H, Kantarci A, Deady J, Hasturk H, Liu H, Alshahat M, Van Dayke TE. Platelet-rich plasma: Growth factors and pro-and anti-inflammatory properties. J Periodontol. 2007;78:661–669.
84. Bendinelli P, Matteucii E, Dogliotti G et al. Molecular Basis of Anti-Inflammatory Action of Platelet-Rich Plasma on Human Chondrocytes: Mechanisms of NF-kB Inhibition Via HGF. J. Cell. Physiol. 2010;225: 757–766.
85. Amable PR, Teixeira MV, Carias RB et al. Mesenchymal Stromal Cell Proliferation, Gene Expression and Protein Production in Human Platelet-Rich Plasma-Supplemented Media. PLoS One. 2014 Aug 12;9(8):e104662
86. Willemsen JC, Spiekman M, Stevens HP et al. Platelet-Rich Plasma Influences Expansion and Paracrine Function of Adipose-Derived Stromal Cells in a Dose-Dependent Fashion. Plast Reconstr Surg. 2016 Mar;137(3):554e-565e
87. Griffeth RJ, Garcia-Parraga D, Mellado-Lopez M et al. Platelet-Rich Plasma and Adipose-Derived Mesenchymal Stem Cells for Regenerative Medicine-Associated Treatments in Bottlenose Dolphins (Tursiops truncatus). PLoS One. 2014 Sep 24;9(9):e108439
88. Cervelli, V., Scioli, M.G., Gentile, P., Doldo, E., Bonanno, E., Spagnoli, L.G., and Orlandi, A. Platelet-rich plasma greatly potentiates insulin-induced adipogenic differentiation of human

adipose-derived stem cells through a serine/threonine kinase Akt-dependent mechanism and promotes clinical fat graft maintenance. Stem Cells Transl Med. 2012;1,206.

89. Liao HT, James IB, Marra KG et al. The Effects of Platelet-Rich Plasma on Cell Proliferation and Adipogenic Potential of Adipose-Derived Stem Cells. Tissue Eng Part A. 2015 Nov;21(21-22):2714-22
90. Kinzebach S, Dietz L, Kluter H et al. Functional and differential proteomic analyses to identify platelet derived factors affecting ex vivo expansion of mesenchymal stromal cells. BMC Cell Biol. 2013 Oct 30;14:48
91. McLaughlin M, Gagnet P, Cunningham E et al. Allogeneic Platelet Releasate Preparations Derived via a Novel Rapid Thrombin Activation Process Promote Rapid Growth and Increased BMP-2 and BMP-4 Expression in Human Adipose-Derived Stem Cells. Stem Cells Int. 2016;2016:7183734
92. Kocaoemer A, Kern S, Klüter H et al. Human AB Serum and Thrombin-Activated Platelet-Rich Plasma Are Suitable Alternatives to Fetal Calf Serum for the Expansion of Mesenchymal Stem Cells from Adipose Tissue. STEM CELLS 2007;25: 1270–1278
93. Borghese C, Agostini F, Durante C et al. Clinical-grade quality platelet-rich plasma releasate (PRP-R/SRGF) from CaCl2-activated platelet concentrates promoted expansion of mesenchymal stromal cells. Vox Sanguinis (2016) 111, 197–205
94. Kølle SF, Fischer-Nielsen A, Mathiasen AB, et al. Enrichment of autologous fat grafts with ex-vivo expanded adipose tissue derived stem cells for graft survival: A randomised placebo controlled trial. Lancet 2013;382:1113–1120.
95. Fukaya Y, Kuroda M, Aoyagi Y et al. Platelet-rich plasma inhibits the apoptosis of highly adipogenic homogeneous preadipocytes in an in vitro culture system. Exp Mol Med. 2012 May 31;44(5):330-9
96. Li K, Li F, Li J et al. Increased survival of human free fat grafts with varying densities of human adipose-derived stem cells and platelet-rich plasma. J Tissue Eng Regen Med 2017; 11: 209–219.

97. Cho HS, Song IH, Park SY, Sung MC, Ahn MW, et al. (2011) Individual variation in growth factor concentrations in platelet-rich plasma and its influence on human mesenchymal stem cells. Korean J Lab Med 31: 212–218.
98. Bernardo ME, Avanzini MA, Perotti C, Cometa AM, Moretta A, et al. Optimization of in vitro expansion of human multipotent mesenchymal stromal cells for cell-therapy approaches: further insights in the search for a fetal calf serum substitute. J Cell Physiol. 2017 211: 121–130.
99. Hsu CW, Yuan K, Tseng CC (2009) The negative effect of platelet-rich plasma on the growth of human cells is associated with secreted thrombospondin-1. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 107:185–192
100. D'Esposito V, Passaretti F, Perruolo G et al. Platelet-Rich Plasma Increases Growth And Motility Of Adipose Tissue-Derived Mesenchymal Stem Cells And Controls Adipocyte Secretory Function. J Cell Biochem. 2015 Oct;116(10):2408-18
101. Chignon-Sicard B, Kouidhi M, Yao X et al. Platelet-rich plasma respectively reduces and promotes adipogenic and myofibroblastic differentiation of human adipose-derived stromal cells via the TGFβ signalling pathway. Sci Rep. 2017 Jun 7;7(1):2954
102. Valina C, Pinkernell K, Song YH, et al. Intracoronary administration of autologous adipose tissue derived stem cells improves left ventricular function, perfusion and remodeling after acute myocardial infarction. Eur Heart J. 2007;28:2667–2677.
103. Man Y, Wang P, Guo Y et al. Angiogenic and osteogenic potential of platelet-rich plasma and adipose-derived stem cell laden alginate microspheres. Biomaterials. 2012;33:8802-11
104. Xiong BJ, Tan QW, Chen YJ et al. The Effects of Platelet-Rich Plasma and Adipose-Derived Stem Cells on Neovascularization and Fat Graft Survival. Aesth Plast Surg (2018) 42:1–8
105. Stessuk T, Puzzi MB, Chain EA et al. Platelet-rich plasma (PRP) and adipose-derived mesenchymal stem cells: stimulatory effects on proliferation and migration of fibroblasts and keratinocytes in vitro. Arch Dermatol Res (2016) 308:511–520
106. Park HB, Yang JH, Chung KH (2011) Characterization of the cytokine profile of platelet rich plasma (PRP) and PRP-induced cell proliferation and migration: Up regulation of matrix metalloproteinase-1 and -9 in HaCaT cells. Korean J Hematol 46:265–273

107. Pierce GF, Mustoe TA, Altrock BW et al. Role of Platelet-Derived Growth Factor in Wound Healing. J Cell Biochem. 1991 Apr;45(4):319-26

108. Hye Kim J, Gyu Park S, Kim WK et al. Functional regulation of adipose-derived stem cells by PDGF-D. Stem Cells. 2015 Feb;33(2):542-56

109. Uhl E, Rösken F, Sirsjö A, Messmer K. Influence of platelet-derived growth factor on microcirculation during normal and impaired wound healing. Wound Repair Regen 2003;11:361–367

110. Rees, R. S., Robson, M. C., Smiell, J. M., and Perry, B. H. Becaplermin gel in the treatment of pressure ulcers: A phase II randomized, double-blind, placebo-controlled study. Wound Repair Regen. 7: 141, 1999.

111. Bachmeier, M., and Loffler, G. The effect of platelet-derived growth factor and adipogenic hormones on the expression of CCAAT/enhancer-binding proteins in 3T3-L1 cells in serum-free conditions. Eur J Biochem 243, 128, 1997.

112. Staiger, H., and Loffler, G. The role of PDGF-dependent suppression of apoptosis in differentiating 3T3-L1 preadipocytes. Eur J Cell Biol 77, 220, 1998.

113. Onogi Y, Wada T, Kamiya C et al. PDGFRb Regulates Adipose Tissue Expansion and Glucose Metabolism via Vascular Remodeling in Diet-Induced Obesity. Diabetes 2017;66:1008–1021

114. Kim JH, Park SG, Song SY et al. Reactive oxygen species-responsive miR-210 regulates proliferation and migration of adipose derived stem cells via PTPN2. Cell Death Dis 2013;4:e588.

115. Craft, R.O., Rophael, J., Morrison, W.A., Vashi, A.V., Mitchell, G.M., and Penington, A.J. Effect of local, long-term delivery of platelet-derived growth factor (PDGF) on injected fat graft survival in severe combined immunodeficient (SCID) mice. J Plast Reconstr Aesthet Surg 62, 235, 2009.

116. Lopatina T, Bruno S, Tetta C et al. Platelet-derived growth factor regulates the secretion of extracellular vesicles by adipose mesenchymal stem cells and enhances their angiogenic potential. Cell Commun Signal. 2014 Apr 11;12:26

117. Eppley BL, Woodell JE, Higgins J. Platelet Quantification and Growth Factor Analysis from Platelet-Rich Plasma: Implications for Wound Healing. Plast Reconstr Surg. 2004 Nov;114(6):1502-8.

118. Oh JH, Kim W, Park KU et al. Comparison of the Cellular Composition and Cytokine-Release Kinetics of Various Platelet-Rich Plasma Preparations. Am J Sports Med. 2015 Dec;43(12):3062-70

119. Hoeben A, Landuyt B, Highley MS, Wildiers H, Van Oosterom AT, De Bruijn EA. Vascular endothelial growth factor and angiogenesis. Pharmacol Rev. 2004;56(4):549-580.

120. Hanft JR, Pollak RA, Barbul A et al. Phase I trial on the safety of topical rhVEGF on chronic neuropathic diabetic foot ulcers. J Wound Care 2008;1:34–37.

121. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab. 2004;89(6):2548–2556.

122. Fain JN, Madan AK, Hiler ML, Cheema P, Bahouth SW. Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. Endocrinology. 2004;145(5):2273–2282.

123. Qiao J, An N, Ouyang X. Quantification of growth factors in different platelet concentrates. Platelets, 2017; 28(8): 774–778

124. Rodella LF, Favero G, Boninsegna R, Buffoli B, Labanca M, Scarì G, Sacco L, Batani T, Rezzani R. Growth factors, CD34 positive cells, and fibrin network analysis in concentrated growth factors fraction. Microsc Res Tech 2011;74(8):772–777.

125. Lacoste E, Martineau I, Gagnon G. Platelet concentrates: effects of calcium and thrombin on endothelial cell proliferation and growth factor release. J Periodontol. 2003;74(10):1498-1507.

126. Schar MO, Diaz-Romero J, Kohl S et al. Platelet-rich Concentrates Differentially Release Growth Factors and Induce Cell Migration In Vitro. Clin Orthop Relat Res (2015) 473:1635–1643

127. Kobayashi E, Flückiger L, Fujioka-Kobayashi M et al. Comparative release of growth factors from PRP, PRF, and advanced-PRF. Clin Oral Invest (2016) 20:2353–2360

128. Seyhan N, Alhan D, Ural AU et al. The Effect of Combined Use of Platelet-Rich Plasma and Adipose-Derived Stem Cells on Fat Graft Survival. Ann Plast Surg 2015;74: 615–620

129. Jin H, Li D, Wang X et al. VEGF and VEGFB Play Balancing Roles in Adipose Differentiation, Gene Expression, and Function. Endocrinology. 2018, 159(5):2036–2049

130. Roberts AB, Sporn MB, Assoian RK, Smith JM, Roche NS, Wakefield LM, Heine UI, Liotta LA,Falanga V, Kehrl JH, Fauci AS. Transforming growth factor-β: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. Proc Natl Acad Sci USA 1986; 83: 4167-4171.

131. Nall AV, Brownlee RE, Colvin CP, Schultz G, Fein D, Cassisi NJ, Nguyen T, Kalra A. Transforming growth factor beta 1 improves wound healing and random flap survival in normal and irradiated rats. Arch Otolaryngol 1996; 122: 171-7

132. Pastar I, Stojadinovic O, Krzyzanowska A, Barrientos S, Stuelten C, Zimmerman K, Blumenberg M, Brem H, Tomic-Canic M. Attenuation of the transforming growth factor beta-signaling pathway in chronic venous ulcers. Mol Med 2010; 16: 92-101.

133. Kim BC, Kim HT, Park SH, Cha JS, Yufit T, Kim SJ, Falanga V. Fibroblasts from chronic wounds show altered TGF-β signalling and decreased TGF-β Type II receptor expression. J Cell Physiol 2003; 195: 331-336.

134. Okuda K, Kawase T, Momose M, Murata M, Saito Y, Suzuki H, Wolff LF, Yoshie H (2003) Platelet-rich plasma contains high levels of platelet-derived growth factor and transforming growth factor beta and modulates the proliferation of periodontally related cells in vitro. J Periodontol 74:849–857.

135. Kawase T, Okuda K,Wolff LF, Yoshie H (2003) Platelet-rich plasma-derived fibrin clot formation stimulates collagen synthesis in periodontal ligament and osteoblastic cells in vitro. J Periodontol 74:858–864.

136. Qin J, Wang L, Zheng L, Zhou X, Zhang Y, Yang T, Zhou Y. Concentrated growth factor promotes Schwann cell migration partly through the integrin β1-mediated activation of the focal adhesion kinase pathway. Int J Mol Med 2016;37(5):1363–1370.

137. Sundman EA, Cole BJ, Fortier LA. Growth factor and catabolic cytokine concentrations are influenced by the cellular composition of platelet-rich plasma. Am J Sports Med. 2011;39:2135-2140.

138. Fain JN, Tichansky DS, Madan AK. Transforming Growth Factor b1 release by human adipose tissue is enhanced in obesity. Metabolism Clinical and Experimental 54 (2005) 1546–1551

139. Mahdy MAA, Warita K, Hosaka YZ. Effects of transforming growth factor-b1 treatment on muscle regeneration and adipogenesis in glycerol injured muscle. Animal Science Journal (2017) 88, 1811–1819

140. Battegay EJ (1995) Angiogenesis: mechanistic insights, neovascular diseases, and therapeutic prospects. J Med 73, 333–346.

141. Tonnesen MG, Feng X, Clark RA (2000) Angiogenesis in wound healing. J Investig Dermatol Symp Proc 5, 40–46

142. Yang Y, Andresen BT, Yang K, et al. (2010) Association of vascular entothelial growth factor-634C/G polymorphism and diabetic retinopathy in type 2 diabetic Han Chinese. Exp Biol Med 235, 1204–1211.

143. Barrientos S, Stojadinovic O, Golinko MS, et al. (2008) Growth factors and cytokines in wound healing. Wound Repair Regen 16, 585–601.

144. Ortega S, Ittmann M, Tsang SH, et al. (1998) Neuronal defects and delayed wound healing in mice lacking fibroblast growth factor 2. Proc Natl Acad Sci U S A 95, 5672–5677.

145. Ning H, Liu G, Lin G et al. Fibroblast Growth Factor 2 Promotes Endothelial Differentiation of Adipose Tissue-Derived Stem Cells. J Sex Med. 2009 April ; 6(4): 967–979.

146. Lopez JF, Sarkanen JR, Huttala O et al. Adipose tissue extract shows potential for wound healing: in vitro proliferation and migration of cell types contributing to wound healing in the presence of adipose tissue preparation and platelet rich plasma. Cytotechnology. 2018. EPub ahead of print

147. Cao R, Brakenhielm E, Pawliuk R et al. Angiogenic synergism, vascular stability and improvement of hind-limb ischaemia by a combination of PDGF-BB and FGF-2. Nat Med. 2003 May;9(5):604-13

148. Widberg CH, Newell FS, Bachmann AW, Ramnoruth SN, Spelta MC, Whitehead JP, et al. Fibroblast growth factor receptor 1 is a key regulator of early adipogenic events in human preadipocytes. Am J Physiol Endocrinol Metab. 2009; 296:E121–131

149. Zaragosi LE, Ailhaud G, Dani C Autocrine fibroblast growth factor 2 signaling is critical for self-renewal of human multipotent adipose-derived stem cells. Stem Cells. 2006; 24: 2412–2419.

150. Xiao L, Sobue T, Esliger A, Kronenberg MS, Coffin JD, Doetschman T, et al. Disruption of the Fgf2 gene activates the adipogenic and suppresses the osteogenic program in mesenchymal marrow stromal stem cells. Bone. 2010; 47: 360–370.

151. Kim S, Ahn C, Bong N, Choe S, Lee DK (2015) Biphasic Effects of FGF2 on Adipogenesis. PLoS ONE 10(3): e0120073.

152. Rubin, R., and Baserga, R. Biology of disease: Insulin like growth factor receptor, its role in cell proliferation, apoptosis, and tumorigenicity. Lab. Invest. 73:311, 1995.

153. Garten A, Schuster S, Kiess W. The Insulin-Like Growth Factors in Adipogenesis and Obesity. Endocrinol Metab Clin N Am 41 (2012) 283–295

154. Botusan IR, Zheng X, Narayanan S et al. Deficiency of liver-derived insulin-like growth factor-I (IGF-I) does not interfere with the skin wound healing rate. PLoS One. 2018. 13;13(3):e0193084

155. Ghiasi Z, Gray T, Tran P, Dubielzig R, Murphy C, McCartney DL, Reid TW. The effect of topical Substance-P plus insulin-like growth factor-1 (IGF-1) on epithelial healing after photorefractive keratectomy in rabbits. Trans Vis Sci Tech. 2018;7(1): 12

156. Yuksel E, Weinfeld AB, Cleek R, et al. Increased free fat-graft survival with the long-term, local delivery of insulin, insulin-like growth factor-I, and basic fibroblast growth factor by PLGA/PEG microspheres. Plast Reconstr Surg 2000;105:1712e20.

157. Loibl M, Lang S, Hanke A et al. Leukocyte-Reduced Platelet-Rich Plasma Alters Protein Expression of Adipose Tissue–Derived Mesenchymal Stem Cells. Plast Reconstr Surg. 138: 397, 2016

158. Zhang P, Lu J, Jing Y et al. Global epidemiology of diabetic foot ulceration: a systematic review and meta-analysis. Ann Med. 2017 Mar;49(2):106-116

159. J Adeghate, S Nurulain, K Tekes et al. (2017) Novel biological therapies for the treatment of diabetic foot ulcers, Expert Opinion on Biological Therapy, 17:8, 979-987

160. Pradhan L, Nabzdyk C, Andersen ND et al (2009) Inflammation and neuropeptides: the connection in diabetic wound healing. Expert Rev Mol Med 11:e2

161. Shi R, Jin Y, Cao C, et al. Localization of human adipose-derived stem cells and their effect in repair of diabetic foot ulcers in rats. Stem Cell Res Ther. 2016;7(1):155.

162. Kato Y, Iwata T, Morikawa S, et al. Allogeneic transplantation of an adipose-derived stem cell sheet combined with artificial skin accelerates wound healing in a rat wound model of Type 2 diabetes and obesity. Diabetes. 2015;64(8):2723–2734.

163. Nambu M, Kishimoto S, Nakamura S et al. Accelerated Wound Healing in Healing-Impaired db/db Mice by Autologous Adipose Tissue-Derived Stromal Cells Combined With Atelocollagen Matrix. Ann Plast Surg 2009;62: 317–321

164. Shin L, Peterson DA. Impaired Therapeutic Capacity of Autologous Stem Cells in a Model of Type 2 Diabetes. Stem Cells Transl Med. 2012 Feb;1(2):125-35.

165. Cianfarani F, Toietta G, Di Rocco G, Cesareo E, Zambruno G, Odorisio T. Diabetes impairs adipose tissue-derived stem cell function and efficiency in promoting wound healing. Wound Repair Regen. 2013;21:545–53.

166. Cramer C, Freisinger E, Jones RK, Slakey DP, Dupin CL, Newsome ER, et al. Persistent high glucose concentrations alter the regenerative potential of mesenchymal stem cells. Stem Cells Dev. 2010;19:1875–84

167. Ko Z, Turnovcová K, Dubský M, Baranovi L, Holá V, Chudí M, et al. Characterization of human adipose tissue-derived stromal cells isolated from diabetic patient's distal limbs with critical ischemia. Cell Biochem Funct. 2014;32:597–604.

168. Ferrer-Lorente R, Bejar MT, Tous M et al. Systems biology approach to identify alterations in the stem cell reservoir of subcutaneous adipose tissue in a rat model of diabetes: effects on differentiation potential and function. Diabetologia (2014) 57:246–256

169. Kim HK, Kim YJ, Kim JT et al. Alterations in the Proangiogenic Functions of Adipose Tissue–Derived Stromal Cells Isolated from Diabetic Rats. Stem Cells Dev. 2008 Aug;17(4):669-80

170. Scimeca CL, Bharara M, Fisher TK, et al. Novel use of platelet-rich plasma to augment curative diabetic foot surgery. J Diabetes Sci Technol. 2010;4(5):1121–1126.
171. Ma C, Hernandez MA, Kirkpatrick VE, et al. Topical platelet-derived growth factor vs placebo therapy of diabetic foot ulcers offloaded with windowed casts: a randomized, controlled trial. Wounds. 2015;27:83–91.
172. Zhou K, Ma Y, Brogan MS. Chronic and non-healing wounds: the story of vascular endothelial growth factor. Med Hypotheses. 2015;85(4):399–404.
173. Kusumanto YH, van Weel V, Mulder NH, et al. Treatment with intramuscular vascular endothelial growth factor gene compared with placebo for patients with diabetes mellitus and critical limb ischemia: a double blind randomized trial. Hum Gene Ther. 2006;17(6):683–691
174. Hanft J, Pollak R, Barbul A, et al. Phase I trial on the safety of topical rhVEGF on chronic neuropathic diabetic foot ulcers. J Wound Care. 2008;17(1):30–32, 34–37.
175. Blakytny R, Jude EB, Martin Gibson J, et al. Lack of insulin-like growth factor 1 (IGF1) in the basal keratinocyte layer of diabetic skin and diabetic foot ulcers. J Pathol. 2000 Apr;190(5):589–594.
176. Blumenschein AR, Freitas-Junior R, Moreira MA et al. Is the combination of fat grafts and platelet rich plasma effective in rats? Acta Cir Bras. 2016 Oct;31(10):668-674
177. Li F, Guo W, Li K et al. Improved Fat Graft Survival by Different Volume Fractions of Platelet-Rich Plasma and Adipose-Derived Stem Cells. Aesthetic Surgery Journal 2015, Vol 35(3) 319–333
178. Nakamura S, Ishihara M, Takikawa M et al. Platelet-Rich Plasma (PRP) Promotes Survival of Fat-Grafts in Rats. Ann Plast Surg 2010;65: 101–106)
179. Oh DS, Cheon YW, Jeon YR et al. Activated Platelet-Rich Plasma Improves Fat Graft Survival in Nude Mice: A Pilot Study. Dermatol Surg 2011;37:619–625
180. Hersant B, Bouhassira J, SidAhmed-Mezi M et al. Should platelet-rich plasma be activated in fat grafts? An animal study. J Plast Reconstr Aesthet Surg. 2018 May;71(5):681-690
181. Pires Fraga MF, Nashio RT, Ishikawa RS et al. Increased survival of free fat grafts with platelet rich plasma in rabbits. J Plast Reconstr Aesthet Surg. (2010) 63, e818-e822

182. Rodriguez-Flores J, Palomar-Gallego MA, Enguita-Valls AB et al. Influence of Platelet-Rich Plasma on the Histologic Characteristics of the Autologous Fat Graft to the Upper Lip of Rabbits. Aesth Plast Surg (2011) 35:480–486

183. Kim DY, Ji YH, Kim DW et al. Effects of Platelet-Rich Plasma, Adipose-Derived Stem Cells, and Stromal Vascular Fraction on the Survival of Human Transplanted Adipose Tissue. J Korean Med Sci 2014; 29: S193-200

184. Blanton MW, Hadad I, Johnstone BH et al. Adipose Stromal Cells and Platelet-Rich Plasma Therapies Synergistically Increase Revascularization during Wound Healing. Plast Reconstr Surg. 123 (Suppl.): 56S, 2009