PRP as a biotherapy for wound healing

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Chronic wounds features

The biochemical conditions that promotes the chronicity of these lesions is characterized by sustained hypoxia and infections (Tandara and Mustoe 2004). Under this condition, stress response pathway is activated leading to the expression of inflammatory cytokines such as TNF and IL-1 but also, endothelial adhesion molecules (such as immunoglobulin and integrins). Activated neutrophils and macrophages recruited in the wound release MMPs and serine proteases which degrade ECM proteins and cleave GFs (Schreml et al. 2010). Nonhealing wounds contain high levels of MMPs, and an abnormally high MMP/TIMP ratio. Degradation of the ECM generates peptides fragments that leads to positive feedback on the inflammatory process. Protein fragments recruit greater numbers of inflammatory cells to the wound bed via chemotaxis and stimulate them to produce more proteases (Adair-Kirk and Senior 2008). Neutrophils and macrophages also release reactive oxygen species (ROS) such as superoxide, which at high levels cause oxidative damage to lipids, DNA and to the ECM. Oxidative damage stimulates signal transduction pathways leading to enhanced MMP and inflammatory cytokine expression. ROS levels are not neutralized by antioxidants such as nitric oxide (NO) since hypoxic wounds are unable to synthesize molecules with antioxidant roles (Schreml et al. 2010). This positive feedback pattern continues until degradation of the wound tissue occurs at a faster rate than new tissue can be synthesized (Menke et al. 2007). The wound then becomes stuck in an inflammatory state and fails to progress through normal healing. An effective intervention must therefore modify this environment that impedes healing and is essential to induce the reparative phase of healing and shorten the prior inflammatory phase.

Platelet rich plasma for chronic wounds: why?

A role for PRP has been proposed in promoting the healing of chronic wounds such as diabetic and venous ulcers via various mechanisms. The hypoxic and pro-inflammatory biochemical environment that impairs healing in chronic ulcers can be reverted by PRP. The rationale behind this treatment is that Local application of PRP provides supra-physiological concentrations of biologically active substances (mainly growth factors) with the ability to modulate biochemical pathways implicated in inflammation and tissue repair.

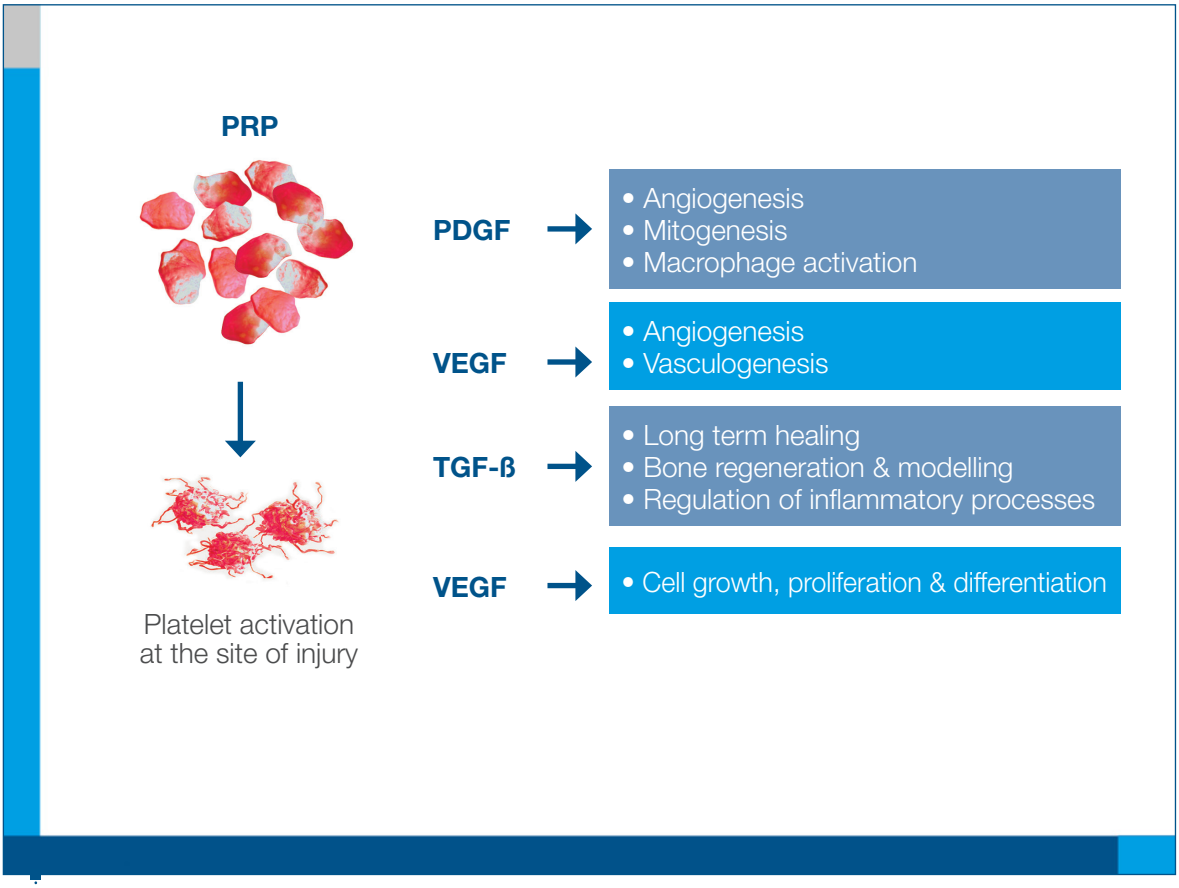

Figure 1. Mechanism of action of PRP. Wound healing and tissue regeneration can be accelerated at the site of tissue injury by various growth factors produced from activated platelets. Abbreviations: PRP, platelet-rich plasma; PDGF, platelet derived growth factor; VEGF, vascular endothelial growth factor; EGF, epidermal growth factor.

Growth factors are biologically active polypeptides that interact with specific cell surface receptors and are very important mediators that regulate the wound healing process. These factors, which are required in high amount to start initial phase of wound healing, primarily promote cell migration into the wound, promote epithelialization, initiate angiogenesis and stimulate the matrix formation and remodeling of the affected area (Werner and Grose 2003). For instance, TGF-1 levels increased in wounds within 24 hours after injury, reduced by 48 hours and peak again at the later stage of healing, promoting collagen deposition (Werner and Grose 2003, O'Kane and Ferguson 1997). VEGF is required to stimulate new vessel formation to meet the higher metabolic demand at the earlier stage of wound healing. VEGF application has been reported with successful results accelerating wound repair by increasing epithelialization, angiogenesis and granulation tissue formation (Enoch,

Grey, and Harding 2006, Hanft et al. 2008). Furthermore, expression of VEGF has been found to decrease after the wound is well granulated as the cellularity and vascularity of wound decline during the remodeling phase (Bao et al. 2009). PDGF is another growth factor up-regulated at the early stage of wound healing (Barrientos et al. 2008) and is involved in chemoattraction of macrophages and fibroblasts for promoting wound healing (Pierce et al. 1989). EGF is secreted by platelets, macrophages and fibroblasts and plays an important role in re-epithelialisation. Brown et al. showed that the topical application of EGF can accelerate epidermal repair in partial-thickness wounds (Brown et al. 1989). In general, PRP induces matrix protein synthesis, matrix remodeling proteins such as MMP-1 and fibroblast proliferation (Kim et al. 2011, Cho, Kim, and Lee 2012). Besides the capacity of stimulating keratinocytes and fibroblasts proliferation, angiogenesis and synthesis and remodelation of extracellular matrix components, PRP can also control inflammation by disrupting NF-B-transactivating activity (Bendinelli et al. 2010).

Platelet-rich plasma in chronic wounds: what do studies conclude?

The use of PRP in preclinical and clinical studies is steadily increasing, and its therapeutic properties in cutaneous wounds have been described in many clinical and experimental studies. There have been several studies addressing the efficacy of PRP in promoting the healing of chronic ulcers experimentally in dogs, rabbits, horses and mice (Chicharro-Alcántara et al. 2018). For instance, Dionyssiou et al. reported healing in skin defects created in the ears of white New Zealand rabbits after applying PRP intralesional more efficiently than the control group (Dionyssiou et al. 2013). Law et al combined human skin cells suspended in platelet rich plasma (PRP) to promote successfully the healing of full-thickness skin wounds in nude mice (Law et al. 2017). Mustoe et al showed that the addition of TGF to incisional wounds in rats accelerated wound healing through increased mononuclear cell infiltration, fibroblast migration and collagen deposition (Mustoe et al. 1987). Farghali et al. tested the effect of perilesional subcutaneous autologous PRP infiltration in full-thickness cutaneous wounds in dogs. Significant increased wound contraction and re-epithelization rate percentages were found tothether with a higher collagen deposition, acceleration of granulation tissue maturation, and a reduced scar formation compared to control wounds (Farghali et al. 2017). Similarly, Jee et al showed that intralesional injection of PRP in acute cutaneous wounds in dogs had a faster healing higher collagen deposition, accelerated re-epithelialization and epithelial differentiation compared to control groups (Jee et al. 2016). Ostvar et al showed faster healing rates, enhanced angiogenesis and adequate granulation tissue formation in full-thickness cutaneous PRP-treated wounds in rabbits (Ostvar et al. 2015).
The main use of PRP in human clinical trials is related to chronic conditions, such as diabetic ulcers, in which the healing is impaired and are characterized by persistent inflammation due to an imbalance between pro-inflammatory and anti-inflammatory cytokines and low growth factor concentration or even due to excess reactive oxygen species (Chicharro-Alcántara et al. 2018). Babaei et al. observed the formation of healthy granulation tissue and early complete closure of every wounds in 150 patients with diabetic foot ulcers after topical application of PRP (Babaei et al. 2017). Hom et al. looked at the healing effects of P-PRP on thigh skin puncture wounds and found that, compared to control, P-PRP-treated wounds closed faster (Hom, Linzie, and Huang 2007). Knigthon et al reported an RCT with 32 patients with chronic lower leg ulcers. At eight weeks re-epithelialization was demonstrated in 81% of PRP treated patients and only 15% of placebo group (Knighton et al. 1990). Chignon-Sicard et al. looked at use of L-PRF in healing of hand wounds. A single L-PRF

application on fresh postoperative hand wounds was associated with faster reepithelialization, with a median improvement of 5 days to the standard treatment of 29 days (Chignon-Sicard et al. 2012). Anitua el at showed that PRP induce better healing in chronic cutaneous ulcers at 8 weeks than standard wound care (mean percentage of surface healed 70 % for PRP while 21 % for the control group; $p<0.05$) (Anitua et al. 2008). Sakata el at, reported 83 % of wound healing within 145 days following PRP injection on 39 patients with with chronic lower extremity wounds of various aetiologies (Sakata et al. 2012). In another series 15 patients with leg ulceration who failed conventional therapy with advanced dressings with duration of lesions, PRP application decreased wound size from a mean of 13.9 to 2.9 cm2 with complete healing of all ulcers within four months (Motolese et al. 2015). Regarding diabetic foot ulcers, Driver et al designed a multicentric clinical trial including 72 lesions. The study results were also better in the PRP group. Complete healing was achieved in 81.3% of the lesions treated with PRP gel, whereas just 42.1% of the lesions in the control group healed (Driver et al. 2006). Cieslik-Bielecka et al, reported enhanced neovascularization and re-epithelialization in AIDS patients with chronic ulcers after topical use of autologous PRP (Cieslik-Bielecka et al. 2018). In contrast to these positive studies, a published meta-analysis including 9 studies with 325 patients concluded that no differences exist regarding wound healing between the PRP group and the control group. This systematic revision entails important limitations, such as the risk of bias in most of the studies and the heterogeneity of the result variables that were measured. Therefore, considering the favorable clinical response found in several studies, the authors suggest that powerful, well-designed clinical trials are needed to determine the real utility of PRP in chronic ulcers healing (Martinez-Zapata et al. 2012). Moreover, variability in the process for obtaining PRP and for applying it, however, may hinder the design and implementation of accurate clinical trials

Platelet rich plasma in chronic wounds: when and how?

Autologous platelet-rich plasma (PRP) has been under development since the 1990s and is increasingly used clinically to treat cutaneous chronic wounds. Even though several studies describe interesting results with PRP application in chronic ulcers, the absence of clinical protocols and guidelines is hindering a more extensive use. As wound repair is a dynamic process, it still remains to be answered whether the delivery of growth factors should be sustained or transient and how long they are required and up to now, no studies have established the most appropriate frequency of PRP application. Concerning methods, PRP application in wounds may be applied intralesional or topical (liquid and gel) or combined. PRP intralesional is usually injected in wound edges in the wound bed. Intralesional application will be limited by the size of the ulcer and the patient's tolerance to pain. Topical application may be combined with intralesional use. Concerning protocol efficacy, so far there are no studies comparing the efficacy of both methods, leaving the best approach to the physician expertise until more high-quality studies gives more lights on this item.

As for conventional an alternatives wounds treatment, efficacy will mainly depend on the presence of a properly prepared wound bed. Previously to PRP application, the wound should be cleaned and adequately debrided. If there exists a high amount of necrotic or unviable tissue, it should be removed and PRP postponed until necessary. If signs of infection are observed, PRP treatment should not be suggested and antibiotic therapy should be administered (Conde-Montero, Dobao, and González 2020).

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