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Wound cicatrisation and platelet rich plasma

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Horses often suffer from chronic non-healing wounds and, conversely, from the development of exuberant granulation tissue (also known as "proud flesh") when wounds are located on the distal limb. Both conditions lead to extensive scarring which may limit a horse's athletic career such that wounds occurring in this location are an important concern to horse owners and veterinarians.
Several mechanisms have been incriminated in problematical repair in horses including poor blood supply,[1] an inefficient inflammatory response to trauma,[2,3] persistent local up-regulation of pro-fibrotic growth factors,[4,5] a disparity between collagen synthesis and lysis[6] as well as microvascular occlusion and deficient apoptosis of the cellular components of granulation tissue[7]. These irregularities are limited to wounds located on the limb while even extensive wounds of the trunk and head usually heal uneventfully.[2,4,8] Wounded skin in horses has been characterized as displaying a weak yet protracted inflammatory response.[2,3] Attempts to ameliorate wound repair in the horse have been disappointing. Indeed, costly treatments are often unsuccessful at preventing or resolving chronic wounds or the development of exuberant granulation tissue.
Bioactive molecule-based therapies comprise one area of tissue engineering that has commanded much attention over the past two decades. A number of cytokines and growth factors, powerful mediators of cell activity, have been isolated.[9] Preliminary studies using a variety of animal models suggested that growth factor therapy might accelerate healing of normal tissues and, especially, promote the repair of impaired wounds. Unfortunately, clinical use of purified bioactive agents has fallen short of expectations.[10] This may relate to the fact that growth factors work in concert, both temporally and spatially, in normal mammalian systems.[11] Because wound repair is a dynamic process, growth factor combination therapy will no doubt be required for efficacy. In an effort to provide this combination therapy, investigators have turned to platelet-rich plasma (PRP).

PLATELET RICH PLASMA (PRP)

PRP represents a concentrated form of multiple growth factors that are released from platelet α-granules at times and locations where platelet plugs are formed; specifically, at sites of tissue injury with hemorrhage and clotting. The growth factors liberated upon platelet degranulation include, among others, platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), and insulin-like growth factor-1 (IGF-1).[9] The principal therapeutic advantage of PRP over isolated purified growth factors is that it represents a physiologically natural mixture of stimulatory and inhibitory mediators designed to have synergistic biologic effects in a wound healing environment.[10]

PRP APPLICATIONS

Platelet Rich Plasma enjoys a wide variety of clinical applications, most prevalently in periodontal, craniofacial and spinal surgeries. The use of PRP has also been reported in the treatment of acute[12] and chronic wounds in man.[13,14,15] In veterinary medicine a preliminary study evaluated the effect of applying a commercially available platelet gel to distal limb wounds in one horse.[16] The heterologous platelet gel reportedly accelerated epithelial differentiation and favored the production of repair tissue showing organized, interlocking collagen bundles. Though a recent study[17] showed that topical apllication of autologous Platelet-Rich-Plasma did not accelerate/improve the quality of repair of small granulating wounds in horses. PRP effects in cartilage, tendons and ligaments have also been reported. *In vitro* studies proved 10% PRP to stimulate chondrocytes to engineer porcine cartilage tissue[18] and 100% PRP to enhance gene expression of the matrix molecules of equine flexor digitalis superficialis tendon with no concomitant increase in the catabolic molecules.[19] A clinical study supports excellent prognosis for returning racing after PRP application in midbody suspensory ligament desmitis associated with controlled exercise.[20]
Topical medications, as opposed to systemically administered drugs, are not required to undergo stringent FDA testing and approval. Consequently, the growth rate for novel technologies in human medicine was of 7.2% between 2000 and 2005. Similarly, a plethora of new products have recently hit the market for equine patients, often with limited scientific evidence supervision to support the company's claims. In an effort to improve the quality of wound management in horses, new methods and technologies should be critically evaluated in order to establish their efficacy, prior to commercialization.

PRP PREPARATION

Technologies to provide autologous PRP are now being used in a wide variety of clinical applications in human medicine. Platelet concentrates can be obtained by means of at least three general methods: the tube (manual), buffy coat (semi-automated) and apheresis (automated) methods. Advantages of the tube method are its low cost and minimal technical requirements. The single and double centrifugation tube method was recently shown to be reliable for concentrating equine platelets and for obtaining potentially therapeutic TGF-β1 levels.[21] In a recent study whole blood was collected atraumatically through a single jugular venipuncture with an 18-gauge needle, in 8 mL citrated anticoagulant platelet sequestration tubes (Fig. 1). Blood was then centrifuged at 3100 rpm for 8 min to achieve separation of cell layers: red blood cells were isolated from the overlying buffy coat and plasma by the gel-like plug within the patented platelet sequestration tubes. Eight mL of whole blood thus yielded approximately 4-5 mL of Platelet Poor Plasma (PPP), of which 80% was discarded. The buffy coat, containing inflammatory cells and platelets, was then gently re-suspended in the remaining 0,75-1.0 mL of PPP to produce PRP. The platelet rich gel is prepared for each treated wound per horse and was activated immediately prior to its application to the wound surface by adding 50 IU of human thrombin reconstituted in 1 mL of $CaCl_2$ (Figg. 2, 3)[17].

PRP IN WOUND CICATRISATION

Autologous platelet gel was recently shown to accelerate epithelialization of full-thickness excisional wounds in human subjects.[12] Specifically, when the platelet count in the gel was more than six times the baseline intravascular platelet count, epithelialization and granulation formation appeared three days earlier. The effect of PRP on wound healing is likely a function of many variables, including the platelet concentration, the volume delivered and the extent and type of injury. It has been suggested that PRP should achieve a three- to five-fold increase in platelet concentration over baseline[9] although the dependence of clinical benefit on platelet concentration versus total number of platelets delivered may need to await further investigation.
TGF-β plays a major role in wound healing through recruitment of macrophages and fibroblasts as well as stimulation of collagen production and inhibition of its degradation via downregulation of matrix metalloproteinases. The TGF-β1 concentration is thought to relate to the platelet count. Alternatively, prolonged expression of TGF-β1 would favor collagen build-up, leading to the development of exuberant granulation tissue. While release of secretory proteins by platelets begins within 10 minutes of clotting, with more than 95% of the presynthesized growth factors secreted within one hour, platelets continue to synthesize and secrete additional proteins for the balance of their life (5-10 days).[9] Moreover, a unique feature of TGF-β is that it can regulate its own production by monocytes and activated macrophages in an autocrine manner, resulting in sustained expression at the wound site following a single exogenous application.[22]
In conclusion, Platelet Rich Plasma has the potential to enhance tissue healing but many more controlled clinical studies are required to establish under which conditions the application of platelet rich plasma is valuable.

Figure 1 - *Venipucnture kit with the 8 mL citrated anticoagulant platelet sequestration tubes.*

Figure 2 - *Platelet Rich Gel preparation after adding 50 IU of human thrombin reconstituted in 1 mL of $CaCl_2$.*

Figure 3 - *Platelet Rich Gel application to a distal limb wound in a horse.*

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