Hemostatic Potential of Platelet-Rich and Platelet-Poor Plasma Utilizing a Partial Thickness Skin Wound Model

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Statement of Purpose: Platelets play a dual role at sites of tissue injury, i.e., healing and hemostasis. The former is mediated by release of biologically-active signaling proteins while the latter is mediated by direct participation in clot formation. Currently, systems are available that produce platelet rich plasma (PRP) from as little as 55-60ml of the patient's blood for wound site application. (1) Platelet poor plasma (PPP), a byproduct of PRP production, has been applied as a hemostatic agent. (1,2) While clinical reports of the use of PRP are generally encouraging, the paucity of controlled clinical studies has made it difficult to substantiate benefit. Controlled animal studies have shown an acceleration of the healing response following PRP application, but have not generally investigated the potential of PRP to function as a hemostatic agent. (3) The purpose of this study was to utilize a porcine partial thickness skin wound model to investigate the hemostatic effects of both PRP and PPP.

Methods: Eight farm pigs (~35-40kg) were used. The protocol was approved by the Institutional Animal Care and Use Committee of the Medical University of South Carolina. Following general anesthesia, 55 ml of venous blood was drawn and combined with 5ml of ACD-A anticoagulant. The blood was then processed using the Gravitational Platelet System™ (GPS II, p/n 800-1001A, Cell Factor Technologies, Warsaw, IN), yielding ~6ml of PRP and ~30ml of PPP. PRP and PPP were each drawn up into 10cc syringes (treatment). Two 1cc syringes were filled with solution consisting of 1000 units of topical bovine thrombin per ml of 10% $CaCl_2$ solution (activation). Treatment and activation syringes were connected, in tandem, to a dual spray apparatus (Micromedics, St Paul, MN) for application to the wound site with the treatment and activation sprays mixed during application to the wound. The wound site was prepared by shaving the back of the animal and creating an 8cm x 6cm partial thickness skin wound (~1mm deep) using a pneumatically-driven dermatome. The wound was preconditioned by observing it for 30s to ensure that a bleeding site was created. After this interval, a cotton sponge was used to absorb the small amount of blood that collected at the wound and this was discarded. Next, the appropriate treatment was applied using the dual sprayer apparatus. The control consisted of no treatment. Following a two-minute interval with the wound uncovered, a preweighed cotton sponge was placed on the wound for three minutes to absorb blood (total 5 minute initial measurement interval). The wet sponge was then weighed so that the mass transfer could be quantified. Another preweighed sponge was immediately placed on the wound for five minutes (2^{nd} measurement interval) and then weighed. This was repeated with one additional preweighed sponge (3^{rd} measurement interval). Also, the iron content of the sponges from three of the animals was measured (acid digestion followed by atomic absorption spectroscopy). Measurements were also made directly on venous blood from these three pigs. The measured blood-iron concentration in these animals (~0.40mg Fe/g blood), coupled with the iron content of the sponges, permitted an independent measure of wound bleeding. Statistical analysis was performed by two-tail paired t-test, with significance taken as $p < 0.05$.

Results / Discussion: Total (15 minute) mass transfer from a given wound was ~1-16g. In the three animals in which iron content was measured, the masses of the wound exudates were greater than the corresponding masses of blood that were calculated. This was likely due to oozing and transfer of interstitial fluid as well as transfer of a portion of the treatment solution to the sponge. (The PRP and PPP treatments contained little or no iron, data not shown). To account for the non-blood mass contained in the sponges, the data from these three animals were used to compute multiplicative correction factors of 0.954, 0.563, and 0.723 for control (no treatment), PRP, and PPP treatments, respectively. These factors were applied to the gravimetric data obtained from the other five animals to provide a more accurate measure of wound bleeding. The combined bleeding over the entire 15 minute period for a given treatment (PPP or PRP) was normalized by dividing it by the control value for that animal. In this way, each animal equally influenced the analysis which was not biased by animals that exhibited profuse or limited bleeding. Table 1 summarizes the results. On a normalized basis, PRP treatment of the skin wounds resulted in about half of the bleeding of untreated controls – a significant difference. Although wounds treated with PPP averaged less bleeding than controls, the difference was not significant. The high variability may account for the lack of a significant hemostatic effect of the PPP treatment. One limitation was that only blood that transferred to the sponge was measured - coagulated blood remaining on the wound was not quantified. However, this study indicates that PRP application can function as an effective hemostat.

Table 1. Comparison of normalized 15 min. bleeding*

	Control	PRP	PPP
Norm. Bleeding	1.00±0.00	0.47±0.29	0.81±0.33
n	8	8	8
P value (compared to control)		0.00125	0.137

*average ± standard deviation

Conclusions: The partial-thickness porcine skin wound model demonstrated the hemostatic effectiveness of PRP treatment but high variability may have been responsible for the neutral effect of PPP. This adds to the body of evidence that supports use of PRP in wound management.

References:

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