Dermatology Clinics & Research
DCR, 3(3): 191-194
www.scitcentral.com

ISSN: 2380-5609

Original Mini-Review: Open Access

Summative Review for Platelet Rich Plasma Combined with a Collagen Scaffold, Measuring *in-vivo* and *in-vitro* Wound Healing Effects

Aaron J. Tabor[1, 3*] and Robert S. Kellar[1, 2]

[*1]Northern Arizona University, Flagstaff, USA

[2]Development Engineering Sciences, Flagstaff, USA

[3]Coconino Community College, Flagstaff, USA

Received November 25, 2017; Accepted January 5, 2018; Published February 23, 2018

REVIEW

Platelet-Rich-Plasma (PRP) is an autologous blood solution, rich in hundreds of growth factors and cytokines that during an inactive state are found in platelet granules. There are a number of commercial companies established that allow clinicians to synthesize and use PRP for orthopedic, cosmetic, intramuscular injections (IM) and wound healing procedures. The premise being that the increased growth factor concentration expedites the desired treatment upon activation and granule exocytosis. PRP is synthesized through a double centrifugation aseptic or sterile process. Robert E. Marx, DDS is credited for being the pioneer of PRP use in oral and maxillofacial surgical procedures and has even defined the working definition of PRP to-date [1].

In this study PRP was synthesized using a modified Messora and Nagata protocol [2]. Porcine acid citrate dextrose (ACD) treated whole blood was purchased from Lampire Biological Laboratories and quantified both manually and via an automated flow cytometry method ensuring an appropriate PRP concentration was produced (**Figure 1**). Based on manual counting methods PRP was appropriately synthesized. Mean platelet count for whole blood was 10.08 ± 3.09 platelets/µl and 878.76 ± 156.28 platelets/µl for PRP ($p < 0.01$). Automated counts found whole blood to be 35,480 ± 6,463.75 and 426,461 ± 47,394 for PRP ($p < 0.01$). These findings established PRP was produced per the Marx definition.

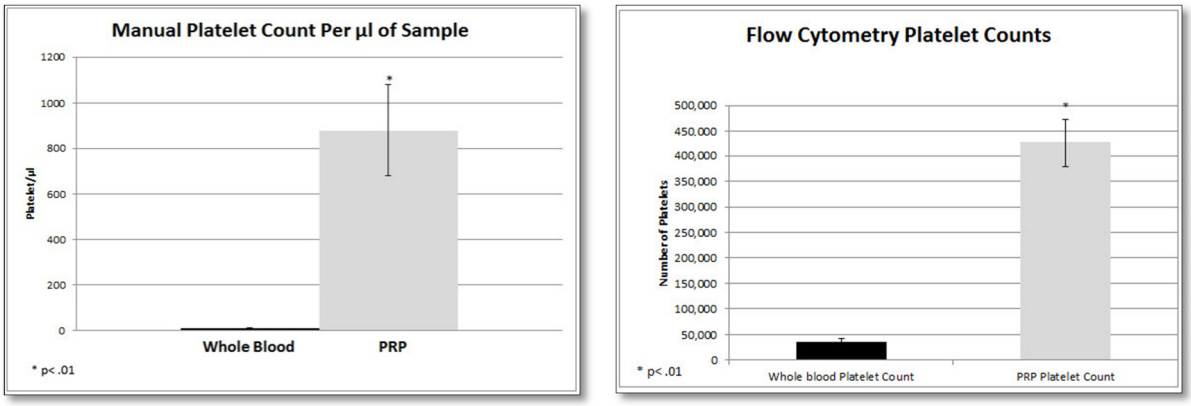

Figure 1. Left graph represents the manual counts and right demonstrates the automated count results. Based on manual counting methods PRP was appropriately synthesized. Mean platelet count for whole blood was 10.08±3.09 platelets/µl and 878.76±156.28 platelets/µl for PRP ($p < 0.01$). Automated counts found whole blood to be 35,480±6,463.75 and 426,461±47,394 for PRP ($p < 0.01$). These findings established PRP was produced per the Marx definition.

Corresponding Author: Aaron J. Tabor, Ph.D., Extended Campuses, Northern Arizona University, Department of Biological Sciences, Northern Arizona University, Math and Sciences Department, Coconino Community College. USA, Tel: 602-725-9505

Citation: Tabor A J & Kellar R S (2018) Summative Review for Platelet Rich Plasma Combined with a Collagen Scaffold, Measuring in-vivo and in-vitro Wound Healing Effects. Dermatol Clin Res, 3(3): 191-194.

Following manual and automated verification visual assessment was performed. Using light microscopy (LM) and scanning electron microscopy (SEM) the PRP was determined to be in an inactive state and was activated on-demand using an electrospun collagen scaffold that was synthesized by the published in-house laboratory protocol [3]. Verification of activated platelets was morphologically validated using prior established literature on pseudopod, bleb formation and platelet clumping. Following morphological verification of inactive and activated forms a bench-top wound healing assay named the scratch assay was implemented to demonstrate the efficacy of the PRP in a wound model prior to mammalian surgical studies. **Figure 2** below demonstrates the SEM morphologic evaluation.

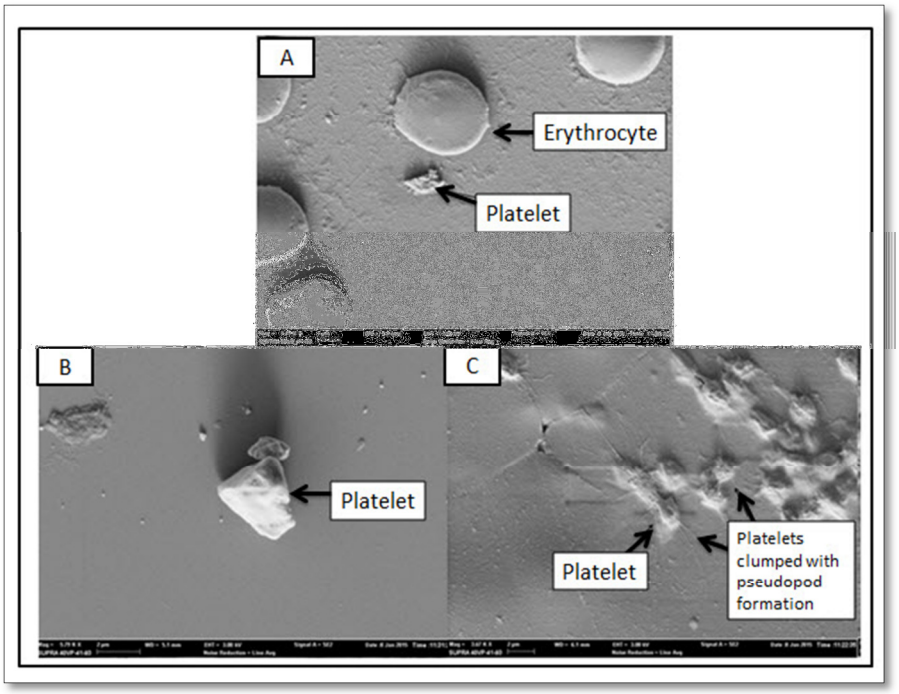

Figure 2. Image A: whole blood with inactive platelet formation. Image B: PRP inactive platelet. Image C: PRP with electrospun collagen scaffold demonstrating the active platelets pseudopod formation and clumping.

Using the scratch assay allows for bench-top validation prior to expensive mammalian studies to occur, this assay acts as a proof of concept and has been validated and established as a useful test in the pre-clinical literature [4]. Initial "scratches" measuring 1.5mm ± 0.5 mm were created in seeded tissue culture confluent monolayers of human neo-natal dermal fibroblast wells with growth media (stock Dulbecos Modified Eagle Medium). Each well was measure for wound closure at four time points (0 4, 8, and 12 hours). Each well was also given a specific concentration of PRP which was able to be viewed using LM for closure assessment. Concentrations of PRP ranged from 0.25%, 0.125%, 0.063%, 0.031%, 0.016% and 0.008% (this experiment was repeated multiple times to increase the sample size). These dilutions were selected as a result of the inability for 100% PRP concentrations to be viewed through the field of view in LM. Upon statistically assessment of percent wound closure in the scratch assay the higher percentages of PRP closed the wounds at earlier time points compared to lower dosages and the control. **Figure 3** below demonstrates the collective findings.

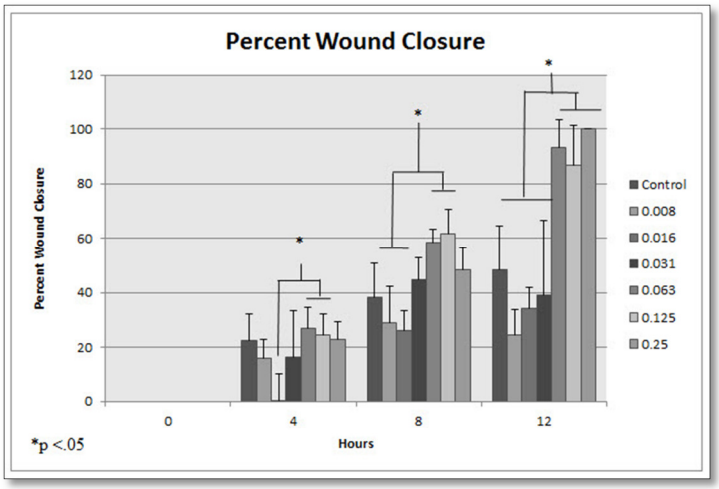

Figure 3. Percent wound closure for in-vitro scratch assay model. Note, statistical significance was demonstrated at three time points, 4, 8, and 12 between higher and lower dosages of PRP and control.

Once bench-top validation had been demonstrated IACUC approval was sought out and granted from Northern Arizona University (NAU) #12-006R1. Using four eight-week old female murine hairless SCID mice a full-thickness wound study was implemented. SCID murine models were selected due to the use of porcine whole blood to synthesize the PRP. The SCID model will negated immunological complications from varying animal tissue sources. Two treatments and a control were applied in the study. The treatments included PRP with an electrospun collagen scaffold (combination therapy) used for platelet activation, a standalone collagen scaffold and lastly standard wound care treatment (sterile gauze and Coban wrap to prevent wound site interference). Assessment of wound sites was performed using photographic images at time points 0, 2, 4 and 6 days. The images were then analyzed with NIH Image J software for percent wound closure. **Figures 4-6** detail the findings at the set time points. At 96 hours post wound creation the combination therapy, PRP with electrospun collagen scaffolds, had a percent closure of 100% compared to the control at 86.076 ± 11.28% (p<0.01). At the same time point, the standalone collagen treatment had a mean closure of 93.15 ± 5.66% and did not demonstrate statistical significance compared to the control (**Figure 5).** The same trends were seen at 144 hours post wound creation with the combination treatment having a percent closure of 100% while the control had a percent closure of 96.48 ± 2.724% (p<0.02). The standalone collagen treatment had a percent closure of 98.741 ± 2.340% and again lacked statistical significance compared to the control.

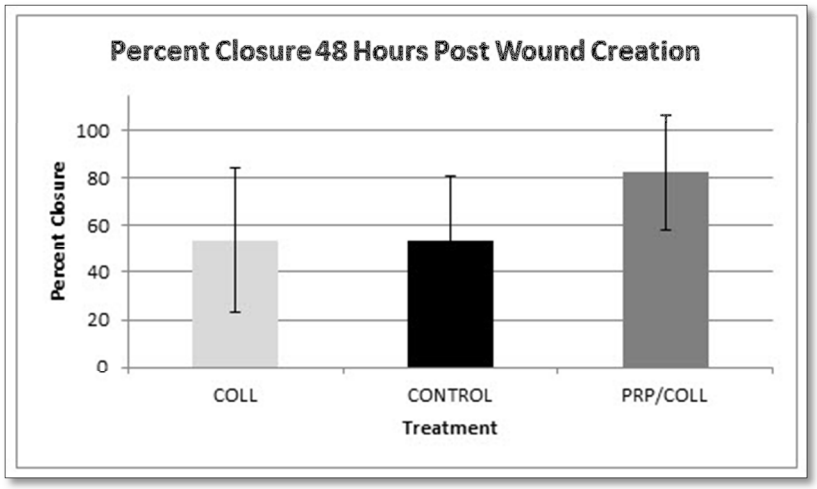

Figure 4. Percent wound closure for *in-vivo* wounds at 48 hours. No statistical significance was present but PRP and electrospun collagen had greater percent closure at 82.73% compared to collagen and control 53.86%, 53.40%, respectively.

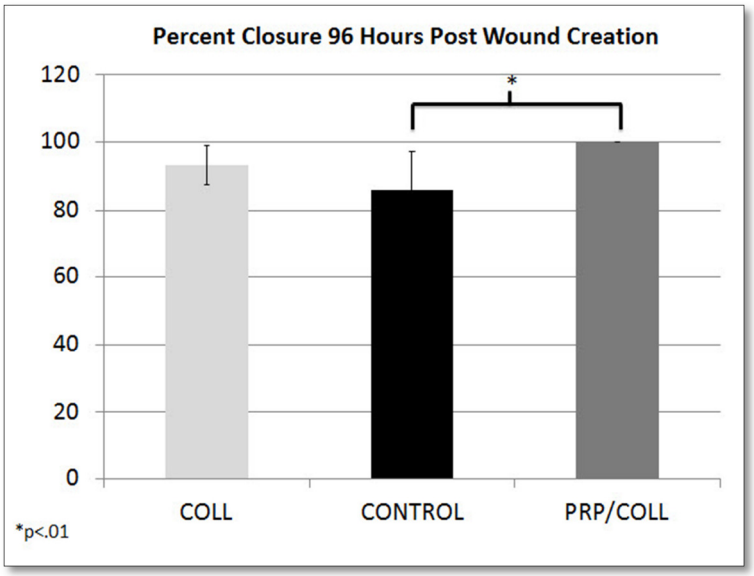

Figure 5. Percent wound closure for *in-vivo* wounds at 96 hours. Statistical significance was demonstrated between control and PRP with the electrospun scaffold.

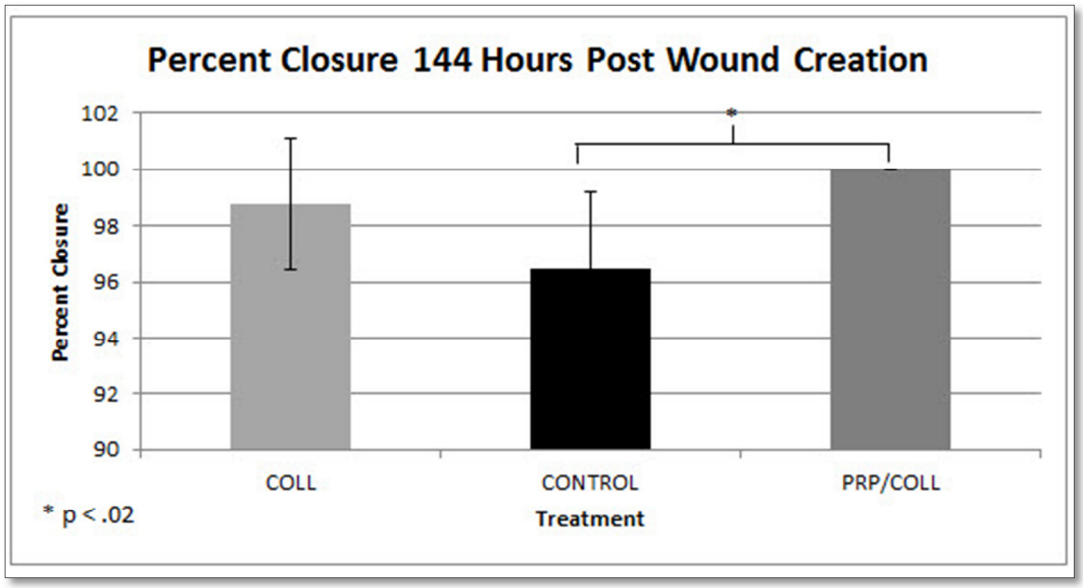

Figure 6. Percent wound closure for *in-vivo* wound at 144 hours. Statistical significance was present between control and PRP with the electrospun scaffold. Note all PRP with electospun collagen treatments were 100% closed compared to control and standalone collagen.

As based on the study results PRP was created, per the Marx definition and PRP does aid in expediting the wound healing process by increasing the percent wound closure within a specified study timeframe (144 hours total). Thiscan subsequently be transferred from a pre-clinical to a human clinical relationship. Furthermore when PRP is combined with an electrospun collagen scaffold activation of the platelets occurs releasing the hundreds of growth factors and proteins into the wound bed. This supports the pre-existing literature that PRP can aid in tissue regeneration as a result of this growth factor concentrate. The ability to delivery extracellular membrane scaffolds, such as the collagen scaffold also aids in the ability to an expedited wound healing event. This happens due to the presence of the extracellular membrane which would normally need to be deposited during the matrix deposition phase of the wound healing cascade, here the membrane is delivered aiding to reduce the potential time needed in that specific deposition phase. In all PRP combined with a collagen scaffold does expedite the wound healing event and can serve as another novel technique to clinically treat full-thickness wounds. Furthermore it may serve as an alternative for patients who may be opposed to traditional treatment modalities. For instance those who may refuse drug intervention or others; the ability to use autologous PRP may eliminate these patient concerns when a clinician is treating the patient with their own body tissues.

DECLARATION

No competing financial interests exist in this study.

REFERENCES

1. Marx RE (2001) Platelet-rich plasma (PRP): What is PRP and what is not PRP? Implant Dent 10: 225-228.
2. Messora MR, Nagata MJH, Furlaneto FAC, Dornelles RCM, Bomfim SRM, et al. (2011) A standardized research protocol for platelet-rich plasma (PRP) preparation in rats. RSBO 8: 299-304.
3. Machula H, Ensley B, Kellar R (2014) Electrospuntropo elastin for delivery of therapeutic adipose-derived stem cells to full-thickness dermal wounds. Adv Wound Care 3: 367-375.
4. Yarrow JC, Perlman ZE, Westwood NJ, Mitchison TJ (2004) A high-throughput cell migration assay using scratch wound healing, a comparison of image-based readout methods. BMC Biotechnol 4.