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Proposing Revisions To The Clinical Platelet Rich Plasma TreatmentFor Future Researches

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Abstract- Healthcare costs continue to rise as do a variety of diseases and disorders that correlate with full-thickness wounds. As a result, finding treatment solutions that areboth effective and cost efficient are important. It is evident in both the current literature and the findings in this dissertation, that platelet-rich plasma has pre-clinical and clinical efficacy. In this paper, the research findings are reviewed, demonstrating that platelet-rich plasma and collagen scaffolds decrease wound healing time. As a result, an amendment to the current clinical PRP definition is suggested due to positive findings that the "on-demand" activation of PRP has in a full-thickness wound. Additionally, the findings of this project will be discussed and how these findings could lead to additional work that may impact PRP treatment modalities in the clinicalfield.

Keywords:Clinical Platelet Rich Plasma, Future studies

INTRODUCTION

Recently published data indicates that the United States spends over $25 billion dollars to treat approximately 6.5 million patient wounds (Sen et. Al, 2009). Here in Northern Arizona, the location of this study, clinicians see numerous cases involving traumatic and full thickness wounds. In fact, recent data supplied by the Northern Arizona Healthcare system demonstrated 1,494 primary admitted wound-patient visits to the local hospital, Flagstaff Medical Center (FMC) between September 2014 and August 2015. Of those patients, 52 underwent amputations and osteotomies, many of which could have been prevented if enhanced therapeutics existed. Many of these wounds require long clinical interventions and standing care, which in turn cause systemic and emotional concerns for patients. As overall health issues related to poor lifestyles choices continue to rise, so do the diseases that correlate with them i.e. diabetes mellitus, malignant cancers and cardiovascular disease (Tanaka & Nakanishi, 1996) (Wang & Widlansky, 2009). Many of these diseases present with a variety of full thicknesswounds that require intensive, continual care from a wound care specialist or surgical team (Armstrong, Wrobel, & Robbins, 2007). It is interesting that many medical advances have progressed over the last 50-100 years but relative wound care management has remained the same. As the need for proper wound care and management follows this "poor health trend" so do cost effective treatment modalities. Fortunately, in the last 20- 25 years, wound care is gaining interest in the public sector and research attention is growing.

Advances such as negative-pressure wound devices (wound VAC's), approved honey wound dressing usage, enzymatic debridement and others have progressed the wound care field since its early years (Venturi, 2005), (Lay-Flurrie, 2008).

Thefurtherdevelopment and enhancement of wound products such as platelet-rich plasma activated with electrospun collagen scaffold therapies could help to reduce these long-term complications, thus improving patient care while making a large economic impact. The average cost to perform a surgical debridement and follow-up wound care can range from hundreds to tens of thousands of dollars depending on the wound location, presence of complications and debridement treatment chosen by the clinicians (Bennett et. al., 2013). The use of platelet-rich plasma and electospun collagen scaffolds not only could resolve these difficult to treat wounds but also reduce the treatment cost. The average cost of platelet-rich plasma production and treatment is between $500.00 and $800.00 dollars. A single electrospun collagen scaffold can cost about $200.00 dollars depending on the collagen composition and type of scaffold. Together a patient would be looking at a treatment that may cost between $700-$1,000.00, far less expensive than the standard and current wound care hospital costs which can range from $4,000.00 to $10,000.00 (Fife, et.at., 2012).

Creating platelet-rich plasma in a cost-effective, non-shearing manner is important because this will preserve the granular contents of the platelets and subsequently prevent the granule cytokine degradation. Many of the current PRP creation modalities are shearing platelets during the production procedure by spinning at extremely high rotations and thus initiating the platelet granule release and subsequent granule degradation process prior to treating the wound. In this project, the PRP was created in an inactive state where no shearing was present; confirmed using light microscopy, SEM and ELISA morphological data findings. The created PRP was then activated using the collagen scaffold, ensuring that the maximum amount of platelet cytokines would be able to be delivered to the wound bed.

These concentrations were determined after an initial failed experiment in which the scratch could not be viewed using the microscope (see figure 1). From these initial pilot experiments, it was determined that a coagulation assay was first needed and subsequently performed to find the PRP ranges in which could be used for the scratch assay.

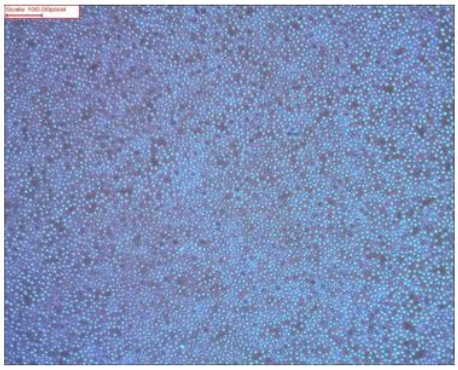

Figure 1: 10x (11/10/14) 100 % PRP; Scratch not visible due to the FOV being saturated with cells

Research Aim

Use the optimized platelet-rich plasma in an *in-vitro* wound healing assay (scratch assay) to expedite the wound healing process.

Research Hypothesis

If an optimized platelet-rich plasma solution is used in a bench-top scratch assay, then it will close the wound faster compared to a control wound withoutPRP.

Materials and Methods: Coagulation Assay

To determine the appropriate platelet-rich plasma concentrations that could be viewed and therefore tested using the scratch assay, a dilution coagulation assay was performed. Starting at 5% PRP concentration and diluting with 10% fetal bovine serum (FBS, Life Technologies) to 0.0078125% PRP it was determined that five unique doses of PRP could be used in the scratch assay; 0.25%, 0.13%, 0.06%, 0.03% & 0.02% (figure 2). This was based on the visibility of the scratch in the microscope field of view (FOV).

Figure 2: Assay performed from 5% and serial diluted down to 0.0078125% PRP. Mixed with Fetal Bovine Serum

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(FBS) (5 unique doses selected for use 0.25%, 0.13%, 0.06%, 0.03% & 0.02%).

Materials and Methods: ELISA

Whole blood and platelet-rich plasma samples were activated with a 10% $CaCl_2$ solution (n= 3 96-well plates). Following platelet activation, the samples were prepared and ran according to MyBioSource Inc. specifications. The samples were analyzed using a BioTEK EON plate reader at 450nm with BioTek Gen5 2.0 all-in-one microplate reader software.

Results

The ELISA showed an increase in growth factors for the activated platelet-rich plasma compared to that of the inactive sample with mean VEGF-A concentrations of 25.297pg/ml and 13.049pg/ml, respectively, as shown in figure 16. Both the SEM results and the ELISA detailed that the creation process is not activating the PRP and that the PRP can be activated on demand, when desired. Although a platelet concentration three-fold abovebaseline is an important aspect of the definition, a process that leaves the platelets in an inactivated state may be even more important in order to have the vesicles deliver the highest concentration of growth factors and cytokines to the wound site when desired.

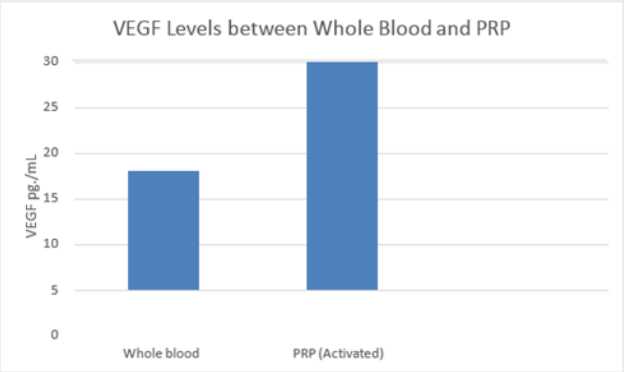

Figure 16: VEGF-A concentrations from samples collected after the PRP creation process and following PRP activation.

Future Study Suggestions

Since the platelet-rich plasma inception in the late 1980's, it has grown to be a widely used treatment modality for a variety of clinical fields. Sheth's group (2012) reported that the platelet-rich plasma market in 2009 was worth 45 million and by the end of 2016 will be worth more than 120 million dollars. With this fast-growing trend, many pre-clinical scientific studieshave been left undiscovered or yet to be thoroughly researched (Sheth et. al., 2012). Here a number of ideas are brought forth as a means to progress the platelet-rich plasma research work.

One study could employ what the mechanism of action is for the expedited wound healing response due to platelet-rich plasma treatment. To date, there are hundreds of cytokines and growth factors released from the granules in a given platelet (Coppinger, Cagney & Toomey et al, 2004). Determining the action of each individual growth factor/cytokine is an important aspect to determine why PRP works as a therapeutic. Historically, this is how many molecular techniques were utilized via ELISA or other assays that employ a single cytokine identification system (Leng et.al., 2008). This would take a significant amount of time to accomplish using PRP, but one could employ a multiplex cytokine assay, many of which have been developed over the last 15 years (Leng et. al., 2008).

This could allow one to determine which cytokines or families ofcytokines are decreasing the wound healing time.

Another study could investigate the antimicrobial effects of PRP as it has been suggested to have antimicrobial properties (Drago, et. al., 2013) (Anitua et. al., 2012). The mechanism of action for this antimicrobial effect and the number of bacterial organisms it works on has yet to be thoroughly researched. To explore this question, a pilot study was employed observing the zone of inhibition against two bacterial organisms; *Pseudomonas aeruginosa* (gram-negative) and *Staphylococcus aureus* (gram- positive) (n= 6 for each plate) using PRP as a treatment. Both bacterial organism's are known and well established infectious agents in wounds. These organisms were tested against a positive and negative control for each of the bacteria. The positive controls i.e.known antibiotics that are commonly prescribed included penicillin and streptomycin. The image below demonstrates the plate set-up and the treatments.

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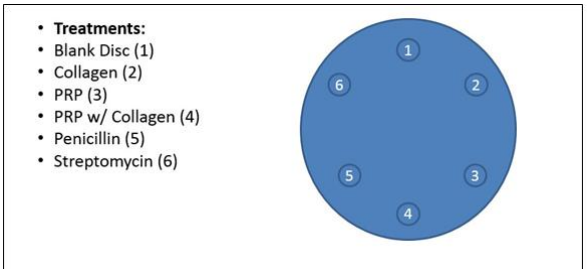

Figure 1: Plate set-up for each bacterial smear. The set-up employed a positive control (antibiotic) and negative control (no treatment) along with activated and inactive PRP.

In observing the bacterial plates over a 72 hour time frame, the data demonstrated that antimicrobial activity was present, but it may not depend on the activated versus inactivate state of the platelet-rich plasma. This is also found in the literature (Anitua et. al, 2012) with respect to various bacterial organisms. Zones of inhibition were observed in some activated forms of platelet-rich plasma and some inactive forms further progressing the question, If PRP is an antimicrobial agent, and if so, why? The plates below demonstrate the study and various zones of inhibition.

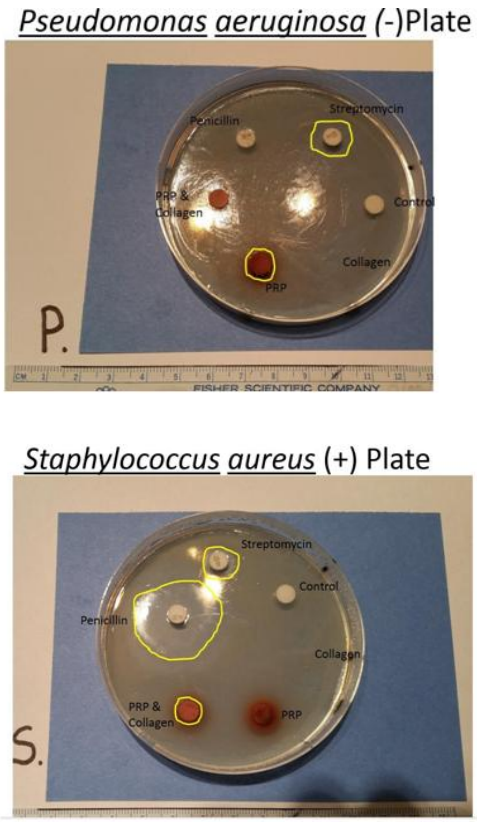

Figure 2: Plates demonstrating the zones of inhibition (in yellow) between the positive controls (antibiotics) and the platelet-rich plasma treatment groups. *P. aeruginosa* is a gram-negative organism and the streptomycin worked as it should have (treats gram-negative bacteria) the penicillin also presented as expected i.e. not treating the *P. aeruginosa*. Note the inactive platelet-rich plasma worked in the *P. aeruginosa* plate but the activated did not. In the gram positive (*S.* aureus) plate the antibiotics worked as expected. The activated PRP created a zone of inhibition the inactive did not.

The findings of the antimicrobial pilot study leave much work to be further completed, but present with some fascinating questions regarding the antimicrobial mechanism of action. In all, future work must be employed to enhance the understanding and acceptance of PRP as a modern cutting edge, cost-effective treatment modality. This future work should include not only larger mammalian models but human trials.

Conclusion

In this paper, platelet-rich plasma was created in an inactive state and in a concentration of platelets three times greater than that of an equal volume of whole blood. The PRP was then activated on-demand using an electrospun type I collagen scaffold. This activation was demonstrated through the use of light microscopy and SEM analysis. Then, using the optimal platelet-rich plasma it was applied to a mock wound, the scratch assay. In this assay, the PRP with the greatest concentration had the greatest percent wound closure compared to the control. The successful demonstration of PRP in the mock wound environment warranted the use of an *in-vivo* wound model system. Using four female SCID mice full-thickness wounds were created on the dorsum and the following treatments were applied; a combination PRP and electrospun collagen scaffold, a stand-alone collagen scaffold and a control. The study findings detailed the greatest percent wound closure with the combination therapy and along with the greatest amount of re-epithelialization and epithelial thickness ratio. Additionally the combination therapy had anatomical evidence of moderate intradermal adipocytes and a reformed stratum corneum, suggesting a further progression of wound healing had occurred compared to the stand-alone collagen and control. This suggested that platelet-rich plasma activated with electrospun

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collagen scaffolds doeseffectively aid in healing full-thickness wounds in a pre-clinical model by decreasing the wound healing time.

References

Armstrong, D. G., Wrobel, J., & Robbins, J. M. (2007). Guest editorial: are diabetes- related wounds and amputations worse than cancer. *Int Wound J*, *4*(4), 286-287.

Arora NS, Ramanayake T, Ren Y, Romanos EG. Platelet rich plasma: A literature review. *Implant Dentistry*. 2009;18(4):303-310

Artitua, E., Andia, I., Ardanza, B., Nurden, P., & Nurden, A. (2004). Autologous platelets as a source of proteins for healing and tissue regeneration. *Thromb Haemost*, *91*(4).

Asaumi, K., Nakanishi, T., Asahara, H., & Takigawa, M. Expression of neurotrophins and their receptors (TRK) during fracture healing. *Bone*, 2000;*26*(6): 625.

Bailey, A. J., Shellswell, G. B., & Duance, V. C. (1979). Identification and change of collagen types in differentiating myoblasts and developing chick muscle. *Nature* 278, 67 – 69

Baker, S. R., Stacey, M. C., Jopp-McKay, A. G., Hoskin, S. E., & Thompson, P. J. (1991). Epidemiology of chronic venous ulcers. *British Journal of Surgery*, *78*(7), 864-867.

Battinelli, E. (2001). Induction of platelet formation from megakaryocytoid cells by nitric oxide. *PNAS*, 98(25), 14458.

Berghoff WJ, Pietrzak WS, Rhodes RD. Platelet-rich plasma application during closure following total knee arthroplasty. Orthopedics. 2006; 29(7):590-598.

Bielecki, T., Gazdzik, T., Arendt, J., Szczeoanski, T., Krol, W., Wielkoszynski, T. (2007). Antibacterial effect of autologous platelet gel enriched with growth factors and other active substances. *Journal of Bone and Joint Surgery*, 89(3): 417-420.

Bluteau D, Lordier L, Di Stefano A, Chang Y, Raslova H, Debili N, Vainchenker W. Regulation of megakaryocyte maturation and platelet formation. *J Thromb Haemost.* 2009;7(Suppl. 1):227–34.

Brem, H., & Tomic-Canic, M. (2007). Cellular and molecular basis of wound healingin diabetes. *The Journal of clinical investigation*, *117*(5),1219-1222.

Crovetti, G., Martinelli, G., Issi, M., Barone, M., Guizzardi, M., Campanati, B., ... & Carabelli, A. (2004). Platelet gel for healing cutaneous chronic wounds.

Transfusion and Apheresis Science, *30*(2), 145-151.

DeLong, M., Russell, R., & Mazzocca, A., "Platelet-rich plasma: the PAW classification system." *Arthroscopy: The Journal of Arthroscopic & Related Surgery* 28.7 (2012): 998-1009.

DeRossi, R., Coelho, A. C. A. D. O., Mello, G. S. D., Frazílio, F. O., Leal, C. R. B., Facco, G. G., & Brum, K. B. (2009). Effects of platelet-rich plasma gel on skin healing in surgical wound in horses. Acta cirúrgica brasileira, 24(4), 276-281.

Di Lullo, Gloria A.; Sweeney, Shawn M.; Körkkö, Jarmo; Ala-Kokko, Leena & San Antonio, James D. (2002). "Mapping the Ligand-binding Sites and Disease- associated Mutations on the Most Abundant Protein in the Human, Type I Collagen". *J. Biol. Chem.277 (6): 4223–4231.*

Donné A. De l'origine des globules du sang, de leur mode de formation et de leur fin. *CR Se Acad Sci* (Paris) 1842;14:366–368

Drago, L., Bortolin, M., Vassena, C., Taschieri, S., & Del Fabbro, M. (2013). Antimicrobial activity of pure platelet-rich plasma against microorganisms isolated from oral cavity. *BMC microbiology*, *13*(1), 47.

Dustin, M. L., Rothlein, R., Bhan, A. K., Dinarello, C. A., & Springer, T. A. (1986). Induction by IL 1 and interferon-gamma: tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). *The Journal of Immunology*, *137*(1), 245-254.

Eppley BL, Woodell JE, Higgins J. Platelet quantification and growth factor analysis from platelet-rich plasma: implications for wound healing. *Plast Reconstr Surg.* 2004;114:1502–1508

Faheem, K. (2014). Part one-Deferential white blood cells count (diff WBCs count) and microscope examination of well stained blood film. *Medicine, science and more.*

Falanga, V. (2005). Wound healing and its impairment in the diabetic foot. *The Lancet*, *366*(9498), 1736-1743.

Foster, T. E., Puskas, B. L., Mandelbaum, B. R., Gerhardt, M. B., & Rodeo, S. A. Platelet-rich plasma from basic science to clinical applications. *The American Journal of Sports Medicine*.2009;*37*(11):2259-2272.

Fennis JP, Stoelinga PJ, Jansen JA. Mandibular reconstruction: a clinical and radiographic animal study on the use of autogenous scaffolds and platelet-rich plasma. *Int J Oral Maxillofac Surg*.2002;31:281-286

Fernández-Barbero, J. E., Galindo-Moreno, P., Ávila-Ortiz, G., Caba, O., Sánchez- Fernández, E., & Wang, H. L. (2006). Flow cytometric and morphological characterization of platelet-rich plasma gel. *Clinical oral implants research*, *17*(6),687-693.

Geddis AE, Kaushansky K. Endomitotic megakaryocytes form a midzone in anaphase but have a deficiency in cleavage furrow formation. *Cell Cycle* 2006; 5:538-45

George J. Platelets. *The Lancet* 2000; 355: 1531-39

Heijnen HFJ, Debili N, Vainchencker W, Breton-Gorius J, Geuze HJ, Sixma J. Mutlivesicular bodies are an intermediate stage in the formation of alpha granules. *Blood* 1998;91:2313-2325

Houghton, P. E., Kincaid, C. B., Campbell, K. E., Woodbury, M. G., & Keast, D. H. (2000). Photographic assessment of the appearance of chronic pressure and leg ulcers. *Ostomy Wound Management*, *46*(4), 20-35.

Hourdille, Paquita, et al. "Thrombin induces a rapid redistribution of glycoprotein Ib-IX complexes within the membrane systems of activated human platelets." *Blood* 76.8 (1990): 1503-1513.

Velnar, Tomaž, Tracey Bailey, and Vladimir Smrkolj. The wound healing process: an overview of the cellular and molecular mechanisms. *Journal of International Medical Research* 37.5 (2009): 1528-1542.

Venturi, M. L., Attinger, C. E., Mesbahi, A. N., Hess, C. L., & Graw, K. S. (2005). Mechanisms and clinical applications of the vacuum-assisted closure (VAC) device. *American journal of clinical dermatology*, *6*(3), 185-194.

Visse, R., & Nagase, H. (2003). Matrix metalloproteinases and tissue inhibitors of metalloproteinases structure, function, and biochemistry. *Circulation research*, *92*(8), 827-839.

Wadhwa, M., Seghatchian, M. J., Lubenko, A., Contreras, M., Dilger, P., Bird, C., & Thorpe, R. (1996). Cytokine levels in platelet concentrates: quantitation by bioassays and immunoassays. *British journal of haematology*, *93*(1), 225-234.

Walter, M. N. M., Wright, K. T., Fuller, H. R., MacNeil, S., & Johnson, W. E. B. (2010). Mesenchymal stem cell-conditioned medium accelerates skin wound healing: an in vitro study of fibroblast and keratinocyte scratch assays. *Experimental cell research*, *316*(7), 1271-1281.

Wang, J., & Widlansky, M. E. (2009). Lifestyle choices and endothelial function: risk and relevance. *Current vascular pharmacology*, *7*(2), 209-224.

White JG, Tubular elements in platelet granules. *Blood* 1968;32:148-156

White JG, Escolar G. Current concepts on platelet membrane response to surface activation. *Platelets* 1993;4:175-198

White JG. Platelet structural physiology: the ultrastructure of adhension, secretion and aggregation in arterial thrombosis. *Cardiovasc Clin*. 1987;18:13-33

White JG. Platelet glycosomes. *Platelets*. 1999;10:242-246

White JG, Gerrard JM. The cell biology of platelets In: Weissman G editor. Handbook of inflammation: the cell biology of inflammation. New York: Elsevier/North Holland; 1980. P. 83-143

Yarrow, J. C., Perlman, Z. E., Westwood, N. J., & Mitchison, T. J. (2004). A high- throughput cell migration assay

using scratch wound healing, a comparison of image-based readout methods. *BMC biotechnology*, *4*(1), 1.

Yeung, E. C. (1999). The use of histology in the study of plant tissue culture systems— some practical comments. *In Vitro Cellular & Developmental Biology-Plant*, *35*(2), 137-143.

Yip, C., Linden, M., Attard, C., Monagle, P., Ignjatovic, V. (2014). Platelets from children are hyper-responsive to activation by thrombin receptor activator peptide and adenosine diphosphate compared to platelets from adults. *British Journal of Hematology, 168,* 526, 532.

Yokota K, Ishida O, Sunagawa T, Suzuki O, Nakamae A, Ochi M. Platelet-rich plasma accelerated surgical angiogenesis in vascular implanted necrotic bone: an experimental study in rabbit. *Acta Orthop*. 2008;79(1):106-110

Zaar, M., Secher, NH., Gam, CM., Rasmussen, P., Johansson, PI. (2011). Interindividual variation in platelets and the cardiovasvular response to heamorrhage in the pig. *Blood Coagulation and Fibronolysis: an internatiol journal in haemostasis and thrombosis*. 22(2), 92-97.