Platelet Redux-Do Concentrated Platelet Preparations have a Place in 21st Century Patient Care?

Deborah McK. Ciombor, PhD
Senior Scientist
Department of Plastic & Reconstructive Surgery
Lifespan Academic Medical Center
The Alpert Medical School of Brown University

Wounds are a costly business in the United States:

Chronic and traumatic wounds represent a significant health problem worldwide. A variety of promising technologies have emerged that have incrementally improved the outcome, however wound healing remains a slow process. Whether we examine acute or chronic wounds costs, they present a significant burden to patients as well as to our society at large. That burden is increasing at an alarming rate. It was calculated, in 2009, that $25 billion was spent on care of chronic wounds in this country[1]. Current estimates indicate that wounds account for almost 4% of total US healthcare system costs. This ever-expanding burden can be explained by the increases seen in our society, of diabetes and obesity, as well as the overall aging of the population.

Acute wounds, resulting from trauma or surgical procedures, represent an additional expansion in healthcare costs. Infection is the most expensive complication following surgical interventions and can result in long term deterioration, leading to chronicity of the wound. The number of inpatient surgeries had risen to 51.4 million by January 2014[2]. Outpatient procedures rose to nearly 42 million over the same time period. Following closely, in dollars spent, is the treatment for scarring that results from many acute wounds.

In 2012, Intellicure, Inc. (a company founded to provide tools for clinicians to optimize patient care) undertook a massive data collection project and calculation. After creating a mathematical model to represent each patient seen in a wound care center, their data showed that regardless of whether the wound healed, the leg was amputated, or the patient was lost to follow up, and regardless of what type of wound the patient had (diabetic foot ulcer or venous stasis ulcer), on average, $5,000 is spent on every patient within the course of care. This is taking into account the charges from the doctor, the cost of the dressings, the home nursing visits, the studies and labs, and the fees from the clinic. Naturally cost varies widely by diagnosis code (wound type) and outcome, but this is a rough average to keep in mind when you see a new patient [3].

Options?

Connective tissue repair is a complex and occasionally an imperfect processs [2, 4, 5]. Clinical and basic research in wound repair is grossly underfunded. Consequently, it remains unknown if chronicity of wounds is due to a perturbation of the normal cascade of repair or due to other intrinsic or extrinsic factors. Nor is it known whether 'normal' wound healing moves forward at an optimal rate. A variety of promising technologies have emerged that have incrementally improved outcomes, however wound healing remains a slow process. Ideally, provisional artificial, skin which could protect and restore structure to the wound in a short time frames allowing cell infiltration and multi-structure regeneration to improve the repair and preempt fibrosis within the fibrin matrix.

William Halsted, M.D., during his career as a John Hopkins surgeon, gained fame for emphasizing strict aseptic technique during surgical procedures. Additionally, he introduced several new operations. Pertinent to our discussion is a radical mastectomy for breast cancer, which he championed. The use of handmade autologous clots as a biomaterial was reported, for use in repair of human mammary defects, by Halsted in 1891 [6]. Past use of platelet concentrates and fibrin clots has been limited, since the time of Halstead, by the difficulty in forming them and inconsistencies in their output.

For nearly three decades, platelet rich plasma, PRP, has been used as an adjuvant to enhance tissue healing, particularly for soft tissues. It has the advantage of being autologous and has been shown to contain several growth factors, in particular TGF-$\beta_{1,}$ VEGF, IGF1 and PDGF-ßß. These growth factors are essential to the body's natural healing process[4, 7, 8]. PRP preparations are said to carry up to 9 times the concentration of platelets, potentially providing an increase in the local concentrations (Figure 1). Numerous studies have indicated positive results with applications of various types of PRP to chronic wounds and bony non-unions. De Leon studied a target population that included 285 chronic wounds (patient n = 200). Wound etiologies included diabetic, pressure, or venous ulcer; dehisced, surgical, or traumatic wound; and wounds of other etiologies. A positive response occurred in 96.5% of wounds within 2.2 weeks with 2.8 treatments. He concluded: "In chronic wounds recalcitrant to

other treatments, utilization of PRP can restart the healing process. Rapid treatment response was observed in 275 of 285 wounds, and the magnitude of response was consistently high, with statistically significant outcomes reported for various subgroups."[9] Therefore, the working hypothesis for PRP use is delivery of a supra-physiologic dose of growth factors and as well as other biologically active agents. However, although PRP has been available for years, its use has proven to be cyclic, moving in and out of vogue among clinicians.

There are significant shortcomings with PRP, most notable are the inconsistent, inefficient and costly production methods. It is dependent on the donor, the technician preparing the product, and the system used to prepare it. Studies establishing clear baselines across product offerings are lacking. While attention is paid to starting platelet counts and dose, issues pertaining to delivery, physical sequestration, microenvironment, and dose kinetics, have been overlooked.[10] This is especially evident when PRP is applied in a wound where fluidic and mechanical factors can prevent portions of the dose from remaining at the intended site long enough to provide the intended dosage. It is then no surprise that few serious studies can be found in the peer reviewed medical or scientific literature.

In addition, most systems require a lengthy, complicated process. Currently, there are at a minimum, 16 commercially available PRP preparation systems, 2 of which are FDA approved. These systems can reach $16,000 in initial cost and each produces a varying quality of product. Currently available commercial systems have a published platelet capture efficiency ranging from 17% to 80% (Table 1).

In addition to inconsistency, PRP production requires, first, the inactivation of the clotting cascade through the use of an anticoagulant. Typically, in the resultant plasma, platelets are activated using bovine thrombin and/or collagen, or less frequently, calcium chloride. The re-initiation of the native clotting cascade is thought to induce the release of growth factors. Unfortunately, growth factors have an exceedingly short half-life once released. Therefore, it is possible, that early in the lengthy process of PRP preparation, there may be a decrease in

bioactivity of any of the growth factors present contributing to the inconsistency of the final product [11].

PRP is so last century…

A novel device developed by Pierce Surgical Instruments in Waterbury, VT, the ClotMaster™ Hula Cup, provides an alternative method to produce a platelet rich, blood-derived, healing adjuvant in solid form. The ClotMaster™ Hula Cup uses autologous whole blood to produce a clot, rich in platelets and encapsulating the same growth factors as those found in clots that form naturally and in PRP. It is a more cost effective and efficient method of preparing a growth factor, platelet rich treatment for wound healing, as it requires no centrifuge or costly production process. By manually swirling a sample of whole blood in the Hula Cup for no more than 10 minutes, a clot is formed. This solid clot can be removed from the Hula Cup and applied directly to the site of a surgical or traumatic wound as a synergistic healing agent, reminiscent of William Halsted's discovery over a century ago.

The ClotMaster™ device consists of a sterile 140 mL polyethylene cup, lid, and sintered glass rod down the center (Figure 2). The height of the glass rod can be adjusted according to manufacturer's specifications to produce different types of clots. ClotMaster clots have preliminarily been shown to capture platelets at a minimum rate of 92%, significantly higher than any commercial PRP system currently on the market. In addition, because the ClotMaster employs mechanical activation of the clotting cascade rather than chemical activation used in typical PRP production methods, inactivated platelets may potentially be preserved in the clot. This would improve the probable long-term bioactivity, essentially acting as an autologous drug delivery system. Additionally, because the biomaterial formed, is either in a dense fibrous form or a solid gel, rather than liquid, these clots can provide a structural bio-scaffold for tissue re-growth (Figure 3). The solid preparation also allows for directed placement by a surgeon, as well as extended contact between the clot and the wound (Figure 4). Liquid PRP contains fibrin, the precursor to fibrinogen monomers; eventually they may form polymeric scaffolds once in place. However as there is no way to ensure that the

liquid PRP remains at the proposed site, an exogenous carrier such as a collagen sponge is required. Overall, the ClotMaster Hula Cup device provides several biological, economical, and practical advantages over traditional centrifuge-derived PRP [12-14].

And what else?

Research, undertaken in the Laboratories of the Department of Plastic and Reconstructive Surgery of the Lifespan Academic Medical Center, is attempting to expand the use of the Clot Master produced biomaterials. In particular, we are using the clots as a platform for tissue engineering soft tissue structures. Other laboratories have shown that exogenously synthesized proprietary fibrin biomaterials may significantly reduce healing times as well as the costs of wound care. Controlled extracorporeal formation of three-dimensional fibrin scaffolds can be used to form tissue inductive custom fillers *in situ* that can act as biodegradable functional soft tissue replacements until the wound bed is rebuilt by local cell infiltration. A fibrin clot also provides extracellular matrix that can readily be assimilated into new structural tissue native to the donor. This potential new class of biomaterial can be used as a primary wound dressing to deliver the growth factors inherent in fibrin, as well as added supplements such as cells, grafts, gene therapies, or drugs [15]. Adhesive, antiseptic, antioxidant secondary dressings comprising high moisture vapor permeability film prevent scab formation by the clot and promote keratinization of the outermost fibrin surface causing a flexible pseudo epidermis to be generated.

Further research, in our laboratories, focuses on the ability of the ClotMaster to provide an ideal bio-similar scaffold for autologous cell seeding. For regenerative surgical procedures, currently dependent on engraftment rate of isolated autologous cell slurry, the potential to deliver a greater number of autologous, stem-like cells in a native scaffold has great advantages. A patient's own adipose-derived adult stem cells can be dispersed in a small volume of their blood prior to processing in the Clot Master, which can then be formed to a custom shape. Tissue engineering and regenerative medicine (TERM) has been dependent, since its inception, upon exogenous, artificial scaffolding. The

potential for the use of a patient-derived, cell-laden, custom-shaped implant provides an exciting 21st century alternative to the unsuccessful tissue engineering attempts of the last century (Figure 5).

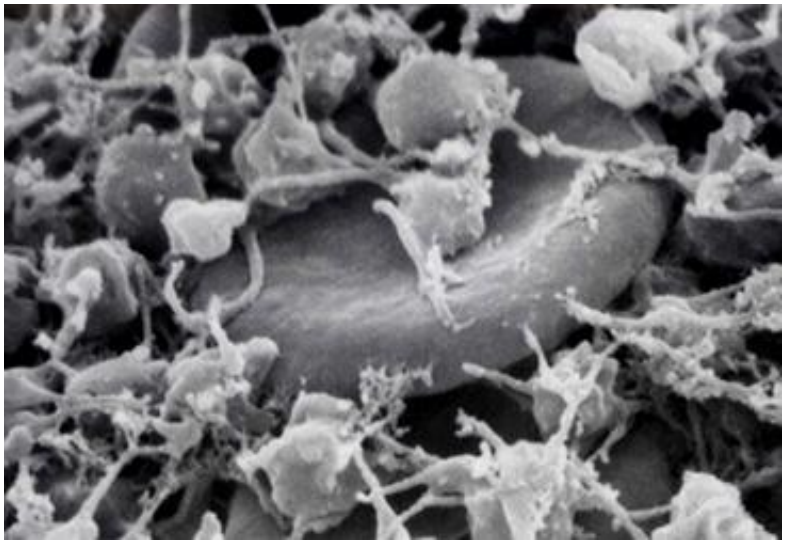


Figure 1

Scanning electron micrograph of a human red blood cell with adherent platelets. The normal platelet count in an adult is 150,000-350,000 per microliter of blood.

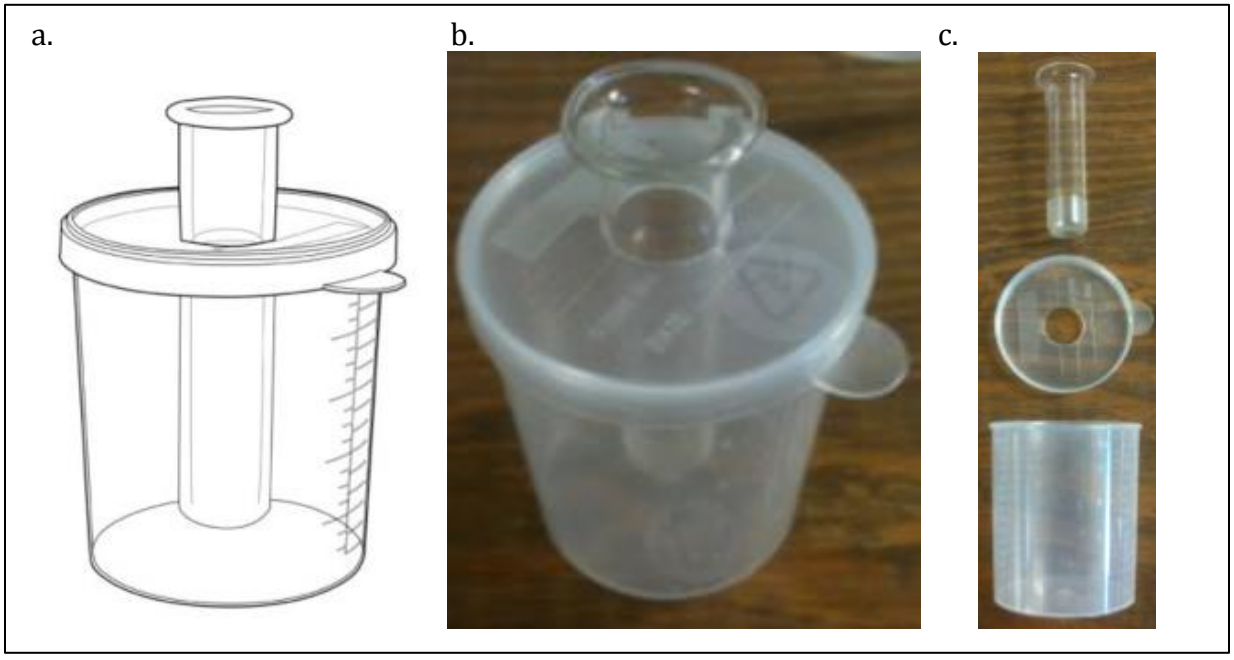


Figure 2

The ClotMaster™ Hula Cup Device: a. ClotMaster™ prototype, b. ClotMaster™ Hula Cup device assemble, c. ClotMaster™ device components disassembled.

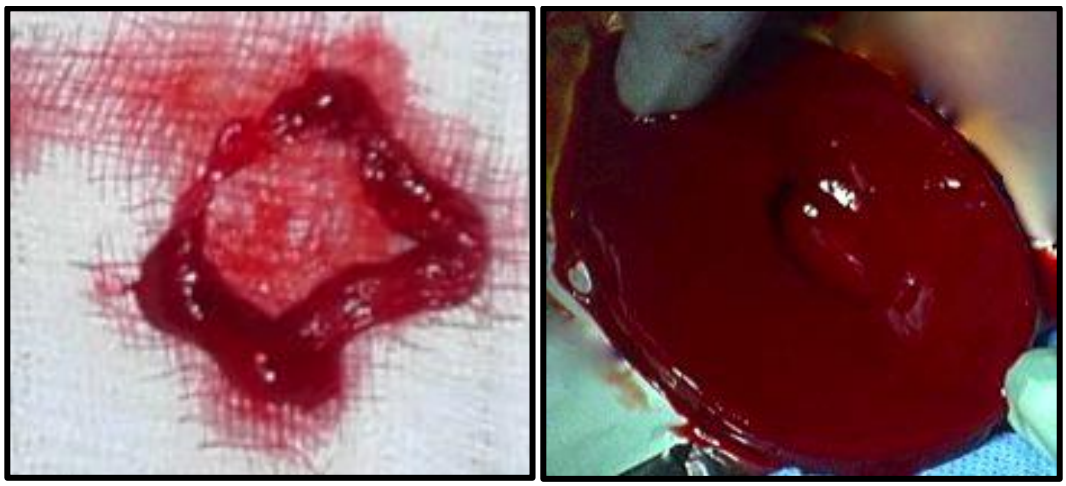
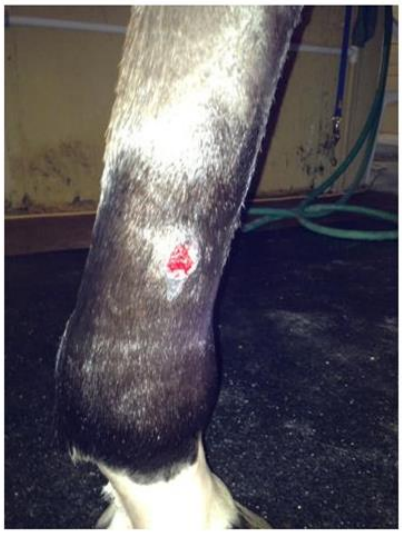


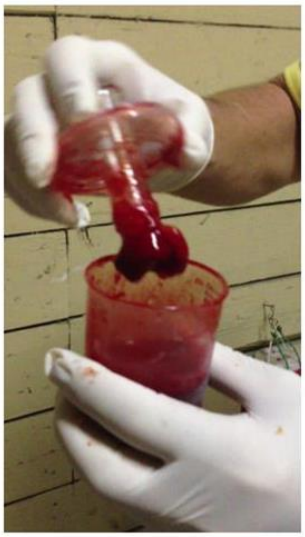
Figure 3

Dense Fibrous and Gel Clot, removed from Hula Cup.

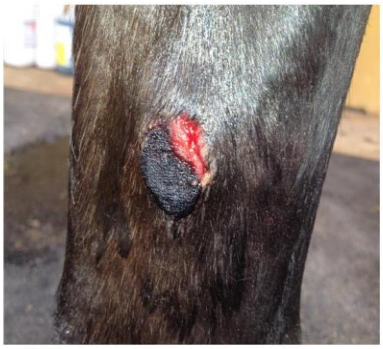
Left: dense fibrous clot removed from Hula Cup and placed on gauze

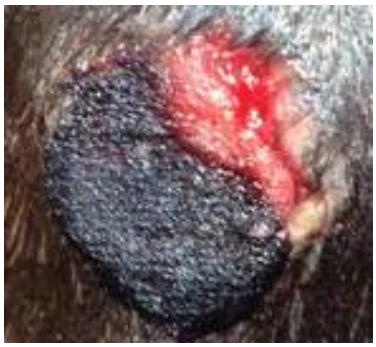
Right: gel clot removed from Hula Cup and held aloft in preparation for placemen



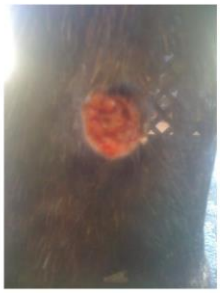


3 Days Post Treatment





6 Days Post Treatment



- The clot filled the defect and provided a robust wound bed that was treated with silver dressings

Figure 4

Veterinary application of dense fibrous clot to a penetrating, infected 3 week old spider bite on a competitive dressage equine patient. 7cc of dense clot was placed into the 3cm deep wound, which healed fully following placement.

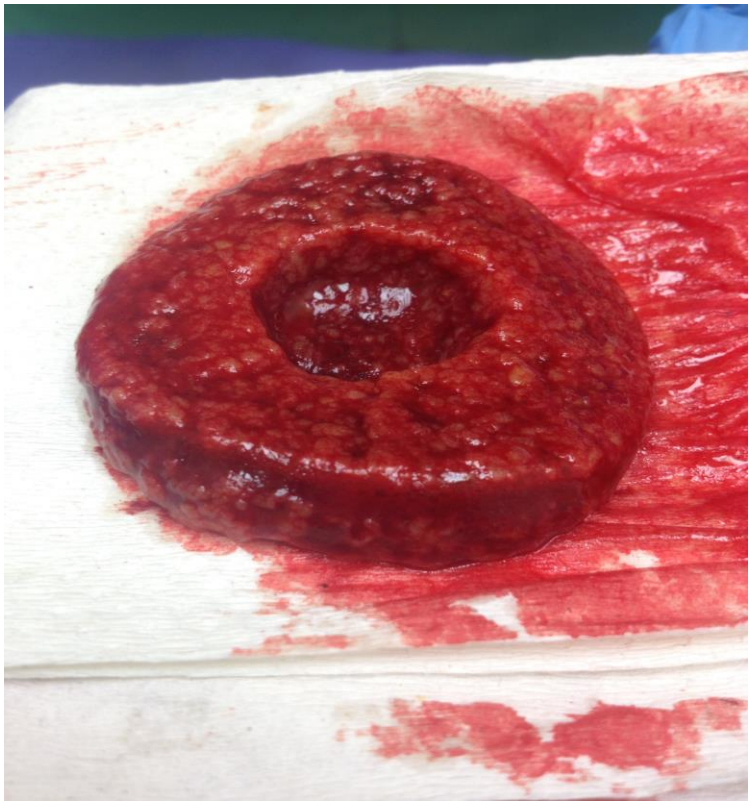


Figure 5

Autologous human gel clot prepared in Clot Master Hula Cup in the operating room. Donor clot carries autologous adipocyte-derived stem cells from lipo-aspiration, dispersed prior to clotting. Preparation was then trimmed and used to augment a breast reconstruction.

Table 1

Sample Platelet Capture Rates for Commercial Systems[6]	
System (company, product)	**Capture Efficiency**
Aesthetic factors, Selphyl (formerly FIBRENET)	66%
Arteriocyte, Magellan	66%
Biomet, GPS III	23-43%
DePuy, Symphony II	70-80%
Emcyte, Genesis	60-70%
Harvest, SmartPReP2	68%
MTF, Cascade	68%
PPAI, Secquire Cell Separator	31%
Stryker/RegenLab, RegenKit-THT	65%
Vivostat, Vivostat PRF	17%
3i Implant Innovations	58-69%

Note: Some of these systems may no longer be available.

1. Walker, D., *The Cost of Outpatient Wound Care.* Today's Wound Clinic, 2009. **3**(4).
2. *FastStats*, N.C.f.H. Statistics, Editor 2013, CDC.
3. Fife, C.E., M.J. Carter, and D. Walker, *Wound Care Outcomes and Associated Cost Among Patients Treated in US Outpatient Wound Centers: Data From the US Wound Registry.* Wounds, 2012. **24**(1): p. 10-17.
4. Guo, S. and L.A. Dipietro, *Factors affecting wound healing.* J Dent Res, 2010. **89**(3): p. 219-29.
5. Buckley, A., et al., *Sustained release of epidermal growth factor accelerates wound repair.* Proceedings of the National Academy of Science, 1985. **82**: p. 7340-7344.
6. Halsted, W.S., *The treatment of wounds with especial reference to the value of the blood clot in the management of dead spaces.* The Johns Hopkins Hospital Reports, 1891. **2**: p. 255–314.
7. Beanes, S.R., et al., *Skin repair and scar formation: the central role of TGF-beta.* Expert Rev Mol Med, 2003. **5**(8): p. 1-22.
8. Foster, T.E., et al., *Platelet-rich plasma: from basic science to clinical applications.* Am J Sports Med, 2009. **37**(11): p. 2259-72.
9. de Leon, J.M., et al., *The clinical relevance of treating chronic wounds with an enhanced near-physiological concentration of platelet-rich plasma gel.* Adv Skin Wound Care, 2011. **24**(8): p. 357-68.
10. Mazzocca, A.D., et al., *Platelet-rich plasma differs according to preparation method and human variability.* J Bone Joint Surg Am, 2012. **94**(4): p. 308-16.
11. Castillo, T.N., et al., *Comparison of growth factor and platelet concentration from commercial platelet-rich plasma separation systems.* Am J Sports Med, 2011. **39**(2): p. 266-71.
12. Fréchette, J.-P., I. Martineau, and G. Gagnon, *Platelet-rich Plasmas: Growth Factor Content and Roles in Wound Healing.* Journal of Dental Research, 2005. **84**(5): p. 434-439.
13. Henderson, J.L., et al., *The effects of autologous platelet gel on wound healing.* Ear Nose Throat J, 2003. **82**(8): p. 598-602.

14. Nagata, M.J., et al., *Influence of the ratio of particulate autogenous bone graft/platelet-rich plasma on bone healing in critical-size defects: a histologic and histometric study in rat calvaria.* J Orthop Res, 2010. **28**(4): p. 468-73.
15. Yang, H.S., et al., *Enhanced skin wound healing by a sustained release of growth factors contained in platelet-rich plasma.* Exp Mol Med, 2011. **43**(11): p. 622-9.