Platelet Rich Fibrin – White Paper

Platelet Rich Fibrin (PRF) is a second-generation blood product where autologous platelets and growth factors, with or without leucocytes, are held in a complex fibrin matrix. PRF has been used to control hemostasis and accelerate the healing of a variety of tissues, including meniscus, periodontal bone, diabetic ulcers, and infected surgical sites.

Unlike the first generation of platelet products, such as platelet-rich plasma (PRP), PRF does not require the addition of an anticoagulant. Instead, whole blood is collected and immediately centrifuged then allowed to clot. The fibrin clot is separated from the red blood cells, compressed on a commercially available medical device and then used as biologic bandage, known as PRF (Figure 1). Depending on the g-forces applied, the clot will contain varying levels of platelets and leukocytes. The centrifuge settings are typically unique for each species. Similarly, PRP can be clotted with the addition of either calcium (as calcium chloride or calcium gluconate) or thrombin. This material is typically referred to as platelet gel rather than PRF. While the membranes may appear similar, PRF has been shown to have greater physical strength that platelet gel and it takes significantly less time to form.

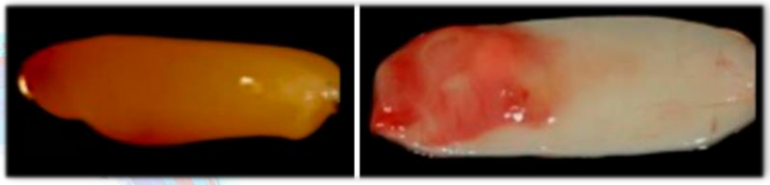

Figure 1. Platelet Rich Fibrin (PRF) as it appears when removed from blood tube (left), and typical appearance of PRF membrane after compression.

When spun optimally, in addition to platelets, PRF contains a multitude of growth factors (Table 1) at concentrations significantly higher than baseline blood. In vitro data supports that a PRF membrane can release growth factors (such as IL-1ra, PDGF and TGF-B) for as long as 21 days. This persistent presence of growth factors is believed to be essential for the angiogenesis as well as the migration and proliferation of cells that is associated with tissue regeneration. In addition to their regenerative properties, platelet concentrates have anti-inflammatory and analgesic effects as well as an antimicrobial effect against a variety of gram positive and gram-negative bacteria (Table 2)

The process that forms PRF, begins with the simultaneous activation of platelets (Figure 2.) and conversion of soluble fibrinogen into fibrin. This cascade begins after endothelial injury when blood comes into contact with molecules such as collagen and Von Willebrand's factor. The fibrin is organized in three-dimensions (Figure 3.) providing strength and elasticity to the matrix. This fibrin network is also able to entrap growth factors and act as a scaffold for cellular migration.

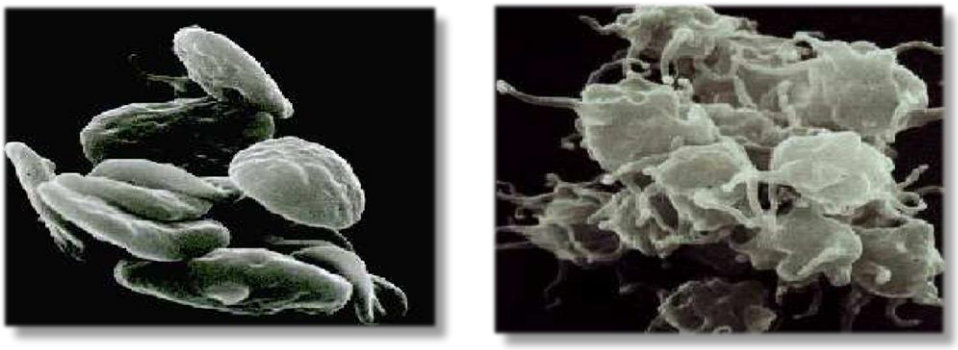

Figure 2. SEM images of unactivated platelets (left) and activated platelets (right)

Rationale for Using PRF: Wounds

The process of wound healing is typically divided into four phases: i) hemostasis, which takes place immediately after injury; ii) inflammatory, leukocytes create an immune barrier against micro-organisms; iii) proliferative, begins within days of injury and lasts for weeks – this phase involves filling in the wound with granulation tissue and blood vessels followed by wound contraction and epithelialization ; iv) remodeling, collagen fibers are reorganized over months providing strength and elasticity to the wound. Every phase of wound healing is regulated by a complex combination of growth factors. PRF not only contains a variety of growth factors that can be released over days to weeks, but it also provides a physical layer of protection as well as a conditional scaffold for the migration of cells.

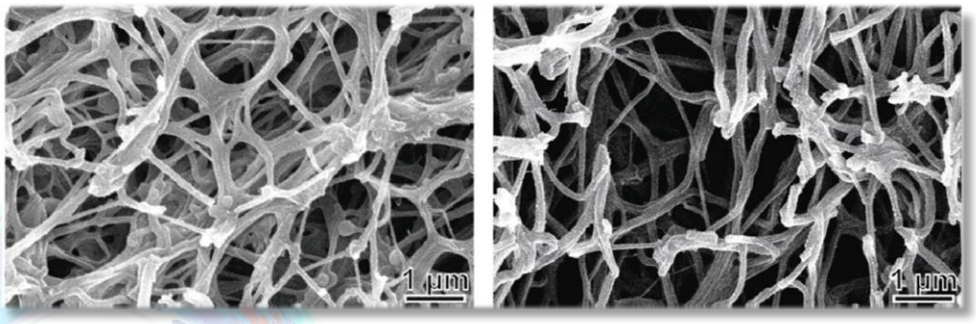

Figure 3. Fibrin network of PRF: closest to RBCs (left), farthest from RBCs (right)

Rationale for Using PRF: Joints

Development and homeostasis of articular cartilage throughout life is regulated by growth factors. PRF has anabolic effects on chondrocytes, synoviocytes and mesenchymal stem cells resulting in increased cellular proliferation and extracellular matrix synthesis, gene expression of chondrocytes proteoglycan and type II collagen synthesis and better cellular organization of cartilaginous tissue.

In Vivo Studies - Animal

- PRF effectively induced endothelial cell proliferation and improved wound angiogenesis in an ischemic/chronic porcine wound model (Roy, 2011)
- PRF was used as a patch for conjunctival lesions in rabbits lead to complete epithelialization (Can, 2016)
- In a murine laminectomy study, PRF significantly reduced epidural fibrosis and chronic inflammatory cell density (Demirel, 2018)
- PRF improved the regeneration of dentin-associated mineral tissue in a canine root canal model (Wang, 2016)
- PRF/aspirin combination was used to significantly enhance periodontal bone formation in a rat model (Du, 2018)
- PRF was used to effectively promote the healing (both macroscopically and histologically superior) of articular cartilage defects of the canine knee (Kazemi, 2015)
- Applied to intestinal anastomosis site, PRF dramatically decreased the local complications and significantly increased the bursting pressures in a rat ischemia/reperfusion model (Ozcay, 2018)

In Vivo Studies – Human

- After other treatment options failed, PRF was successfully used in a kidney transplant-recipient with gangrenous cystitis (Iesari, 2017)

- Meta-analysis showed PRF to be both clinical useful as well as cost effective during sternotomies to reduce infection rates (Kirmani, 2016)
- PRF was shown to be superior to PRP for periodontal regeneration (Sucetha, 2015)
- Alveolar osteitis was reduced 90% when PRF was placed in sockets of patients undergoing bilateral wisdom tooth extraction (Hoaglin, 2013)
- PRF was used in a series of patients to successfully control hemorrhage and heal palatal wounds (Kulkarni, 2014)
- A randomized study involving 20 patients with chronic periodontitis, addition of PRF showed statistically significant improvements in probing depth, clinical attachment and bone fill over Bioactive Glass alone (Bodhare, 2018)
- A study evaluating therapies for nonhealing diabetic foot ulcers found that PRP gel would be the most cost-effective treatment while also providing the highest quality of life (Dougherty, 2008)
- Patients with knee osteoarthritis had better outcomes when PRF was used with microfracture compared to microfracture alone at 5 year evaluation (Papalia, 2015)
- In a case series12 patients had complete radial tears of the meniscus surgically repaired with the addition of PRF, 11 of 12 cases showed complete healing on MRI and 6 of 7 on second-look arthroscopy (Ra, 2013)
- PRF in combination with antibiotics was more effective than hyaluronic acid and antibiotics in a randomized study of 60 patients with infected knee osteoarthritis (Zhang, 2018)

SUMMARY

Platelet Rich Fibrin is an autologous second-generation blood product that is safe, easy to prepare and cost effective. Numerous in vivo studies have demonstrated efficacy for tissue healing in a variety of tissues and injuries in multiple species.

Reasons for using PRF over PRP:

- Avoid added chemicals (anticoagulant/clotting agent)
- Strong desire for biologic bandage
- Strong desire for delayed release of growth factors

Limitations of PRF:

- Need to keep wound moist for optimal results
- May not work if patient is on anticoagulant therapy
- Limited blood availability with small and/or anemic patients

Tips for advanced users:

- If a limited amount of blood is available, say 12cc, it is better to completely fill on blood tube with 10+cc of blood rather than split evenly between two tubes
- The end of the membrane closest to the RBCs will contain more growth factors – in some wounds it may be advantageous to align this end over the most vital structures

Conditions that may affect formation of a normal fibrin clot:

- Acidosis/alkalosis
- Hypochloremia/hyperchloremia
- Blood dyscrasias (e.g. Von Willebrand disease)

Table 1. Selected growth factors found in PRF and some of their properties.

EGF (Epidermal growth factor)	stimulates re-epithelization, angiogenesis, and collagenase activity
bFGF (Fibroblast growth factor, basic)	stimulates angiogenesis, endothelial cell proliferation , collagen synthesis, wound contraction, matrix synthesis, epithelization, keratinocyte growth factor production
aFGF (Fibroblast growth factor, acidic)	potent GF for skin keratinocytes playing role in tissue repair following skin injuries.
KGF (Keratinocyte growth factor)	acts predominantly on epithelial cells to elicit a variety of responses including proliferation, migration and morphogenesis
GM-CSF (granulocyte/macrophage colony-stimulating factor)	stimulates both the proliferation and the differentiated function in osteoblasts
IGF-1 (Insulin-like growth factor)	mediates the action of GH in peripheral tissues, as such it is a major mediator of prenatal and postnatal growth
PDGF (Platelet derived growth factor)	potent mitogens for connective tissue cells, including dermal fibroblasts, arterial smooth muscle cells, chondrocytes and some epithelial and endothelial cells
TGF-ß (Transforming growth factor, beta)	stimulates monocytes to secrete FGF, PDGF stimulates collagen synthesis decrease dermal scarring
VEGF (Vascular endothelial growth factor)	highly specific mitogen for vascular endothelial cells induces neovascularisation under physiological conditions

Table 2. List of microbes inhibited by platelet concentrates.

Bacteria
MRSA (methicillin resistant Staph. aureus) (also reduced biofilm formation)
MSSA ("" sensitive "" "")
MRSE ("" resistant "" epidermis)
MSSE ("" sensitive "" "")
Pseudomonas aeruginosa
Klebsiella pneumoniae
Shigella sp.
Streptococcus epidermis
Streptococcus agalactaie
Streptococcus oralis
Enterococcus faecalis
E. coli
P. acnes
N. gonorrhoeae
Moraxella catarrhalis
Candida albicans

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Figure 2. https://vet.uga.edu/ivcvm/courses/VPAT3100/01_circulation/hemostasis/hemostasis03.html retrieved 021519

Figure 3. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5304401/ retrieved 020919