



THE SCIENCE & EVOLUTION BEHIND PRF

LIQUIDPRF

BIOPRF

SOLIDPRF

The evolution of Platelet Rich Fibrin

Platelet concentrates have been utilized in medicine for over 3 decades owing to their ability to rapidly secrete growth factors. They have gained tremendous momentum as a regenerative agent derived from autologous sources capable of stimulating tissue regeneration in a number of medical fields.^{1,2}

Many years ago, it was proposed that by concentrating platelets utilizing a centrifugation device, autologous growth factors derived from blood could be collected from a platelet-rich plasma layer, and later utilized in surgical sites to promote local wound healing.^{1,2} Today, it has been well established that platelet concentrates act as a potent mitogen capable of:

- ✓ Speeding the revascularization of tissues (angiogenesis)
- ✓ Acting as a potent recruitment agent of various cells including stem cells (chemotaxis)
- ✓ Inducing the prompt multiplication of various cell types found in the human body (proliferation)

Various systematic reviews from multiple fields of medicine have now demonstrated their ability to support the regeneration of a number of cell types and tissues. Below we review the evolution of platelet concentrates.

1990s



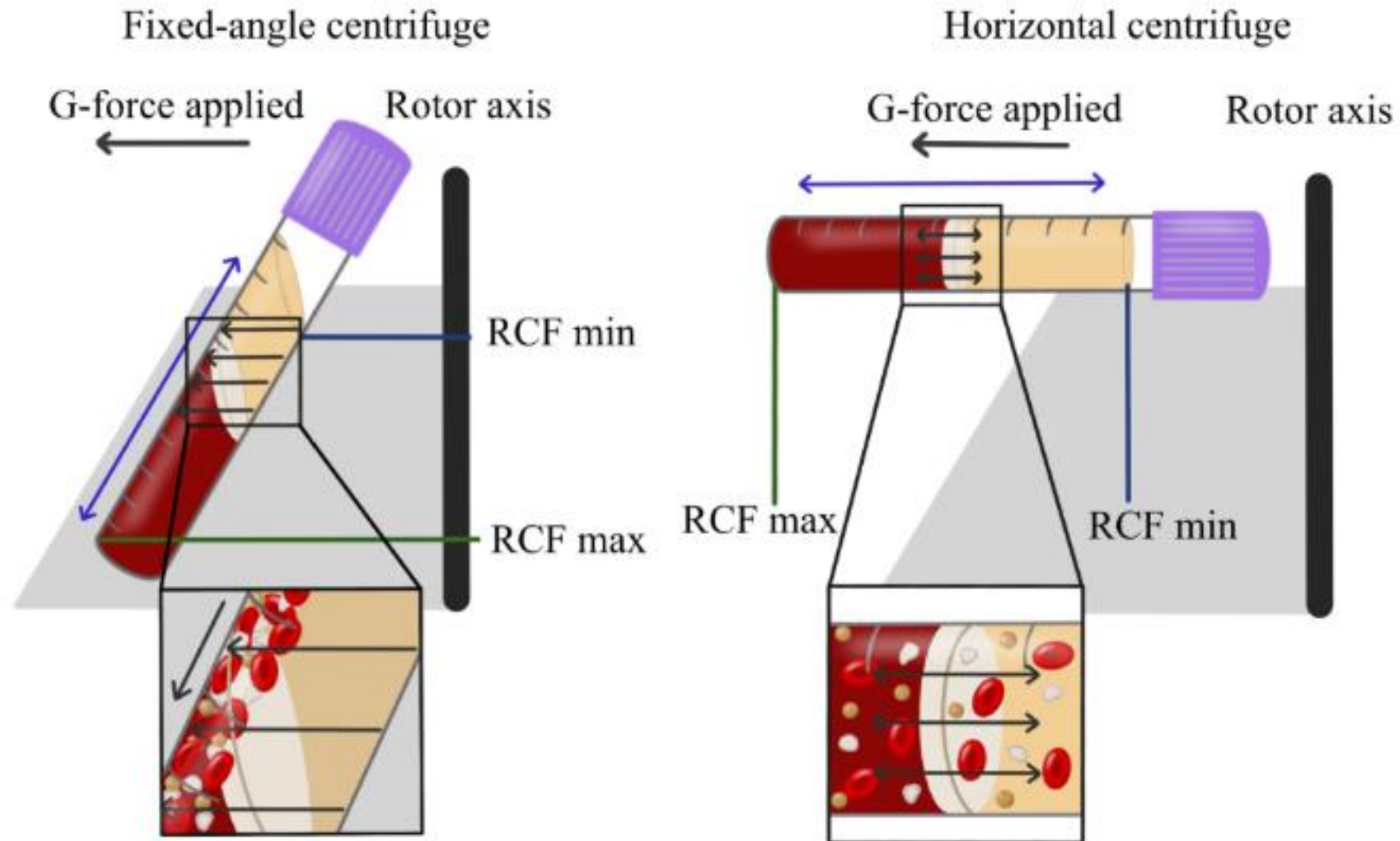
2000-2010



2014-2018



2019 and Forward



Platelet Rich Plasma (1990s)

Work directed by Dr. Robert Marx in the 1990s led to the popular working name 'platelet rich plasma' (PRP). The goal of PRP was to collect the largest and highest concentrations of platelets/growth factors to be later utilized for regenerative purposes. The PRP protocol required extensive centrifugation time (typically over 30 minutes). During this process, the use of anticoagulants (namely bovine thrombin or calcium chloride) was absolutely necessary in order to prevent clotting owing to the lengthy centrifugation times. The final composition of PRP contained over 95% platelets, known cells responsible for the active secretion of growth factors involved in wound healing.

Two reported drawbacks of PRP have since been reported in the literature. First, centrifugation times were deemed long (>30 minutes) and not practical for a variety of surgical procedures that may routinely be performed in many clinical practices. Furthermore, it has since been revealed that clotting in general, is a necessary component of normal physiological wound healing. This limitation of using anti-coagulants prevented optimal and led to the development of platelet rich fibrin.

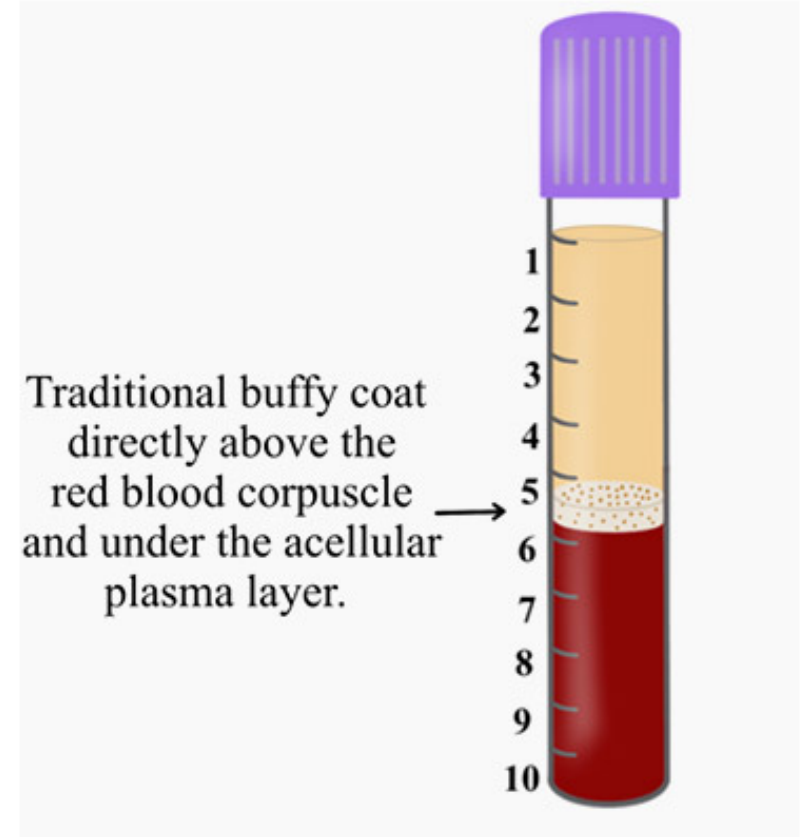


Figure: PRP was introduced with an ability to concentrate platelets within the buffy coat. This was effective because PRP utilized anti-coagulants whereby the majority of cells were found within the buffy coat. Various protocols and equipment have been introduced to isolate these cells but the concept remains that cells within the 'buffy coat' are isolated with use of anti-coagulants.

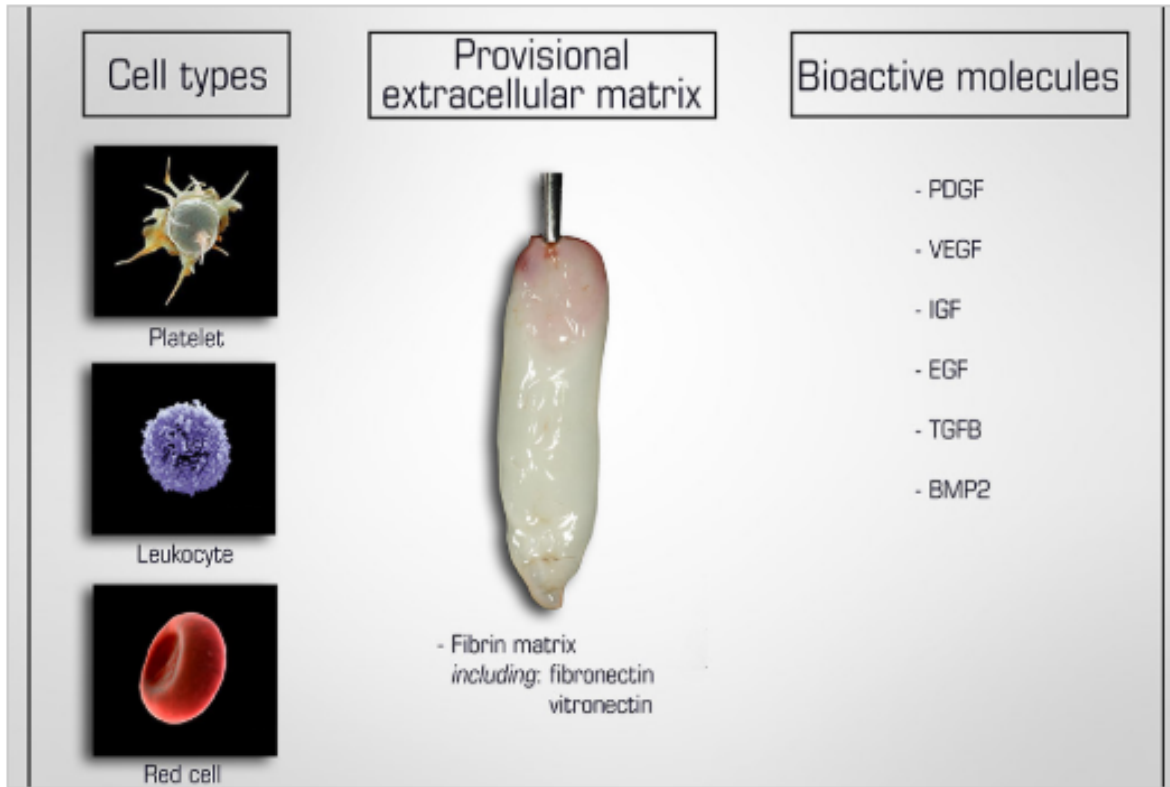


Figure: Natural components of PRF include 1) cell types (platelets, leukocytes and red blood cells), 2) a provisional extracellular matrix 3-dimensional scaffold fabricated from autologous fibrin (including fibronectin and vitronectin) as well as 3) a wide array of over 100 bioactive molecules including most notably PDGF, VEGF, IGF, EGF, TGF- β and small quantities of BMP2

Leukocyte and Platelet Rich Fibrin: L-PRF (2000-2010)

Owing to the drawback that the anticoagulants utilized in PRP prevented clotting, pioneering work led by dr. Joseph Choukroun and dr. David Dohan led to the development of platelet rich fibrin (PRF). The aim of PRF was to develop a second-generation platelet concentrate with anti-coagulant removal. Since anti-coagulants were removed, a much quicker working time was needed and the practitioner absolutely required that centrifugation began shortly thereafter blood draw (otherwise blood would clot). Furthermore, high g-force centrifugation protocols were utilized in order to separate blood layers in attempt to separate blood layers prior to clotting.

The final spin cycle (2700 RPM for 12 minutes = 700g), resulted in a plasma layer composed of a fibrin clot with entrapment of platelets and leukocytes.

Advanced and injectable Platelet Rich Fibrin: A-PRF and i-PRF (2014-2018)

While much of the research performed in the late 2000s and early 2010s was dedicated to the clinical uses and indications of L-PRF, major discoveries were made several years following extensive clinical use of L-PRF. In 2014, an oral maxillofacial surgeon in Germany by the name of Dr. Shahram Ghanaati observed histologically that following centrifugation at high g-forces (~700g – utilized in L-PRF protocols), the majority of leukocytes and platelets were in fact located at the base of L-PRF clots or even worse, within the red corpuscle blood layer at the bottom of centrifugation tubes.

Pioneering research within his laboratory led to the development of an advanced platelet rich fibrin (A-PRF) whereby lower centrifugation speeds (~200g) led to a PRF members with more evenly distributed platelets. these newer protocols more favorably released a higher concentration of growth factors over a 10 day period when compared to PRP or L-PRF.



L-PRF protocol:
2700 RPM for 12 minutes

A-PRF protocol:
1300 RPM for 8 minutes

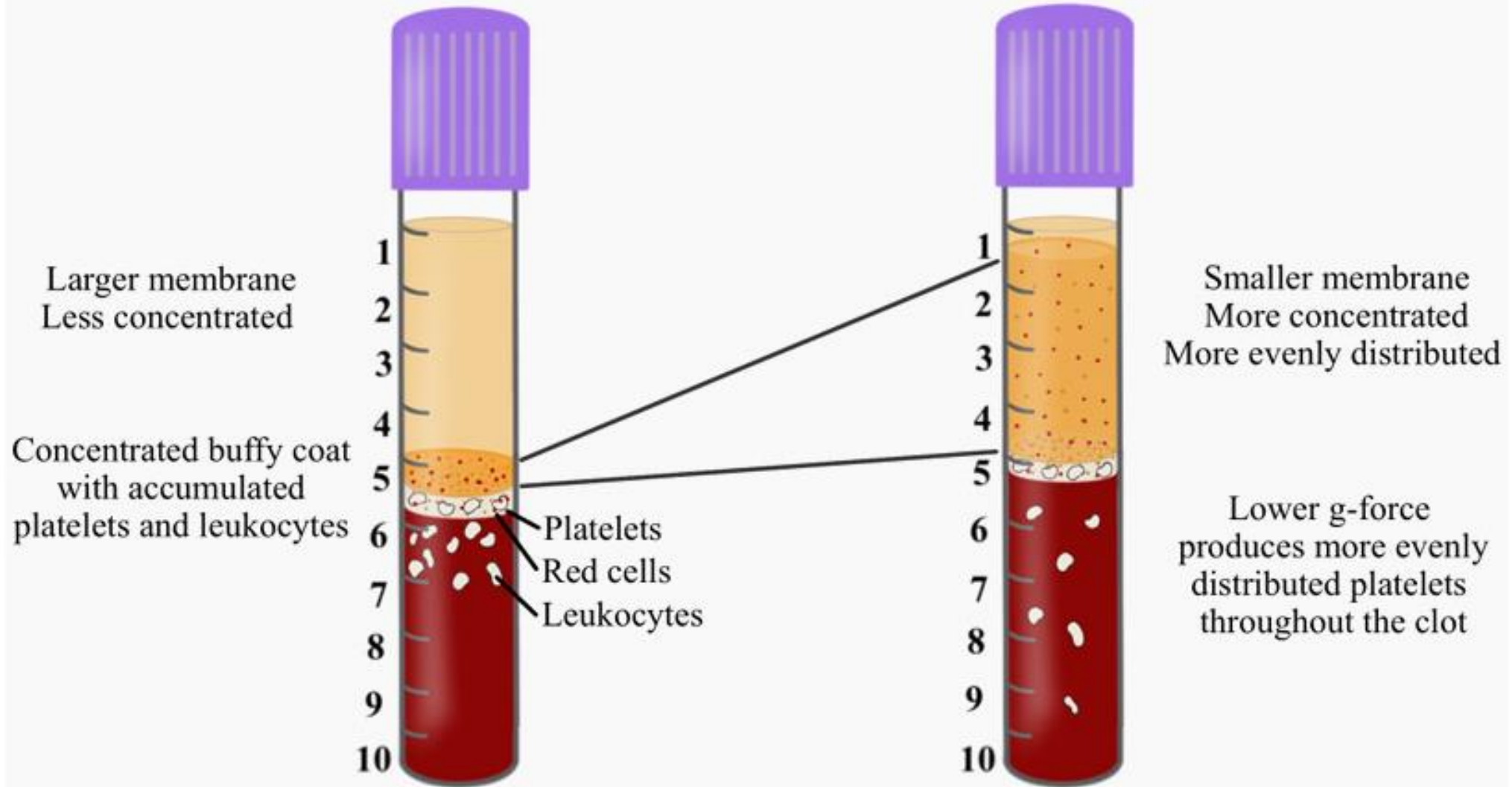


Figure: The effects of both L-PRF and A-PRF protocols on the final outcomes. Since PRF is obtained from the upper layer of PRF tubes, it becomes obvious that the L-PRF protocols generate PRF membranes that are mainly devoid of cells with the largest accumulation observed directly within the buffy coat. In contrast, the A-PRF protocol is able to more evenly distribute cells. This is one of the advantages of utilizing this novel quantification method proposed in a recent article (Miron et al. 2019).

PITFALLS TO THE CURRENT METHODS UTILIZED TO QUANTIFY CELL TYPES IN PRF

Two pitfalls exist with quantifying cell types found within PRF. Histological studies have been infrequently performed and show variability and bias in results. It becomes very difficult to assess where cells are located following cell separation. Commonly, following centrifugation, the plasma layer was then removed and sent for complete blood counts (CBC) as depicted below.

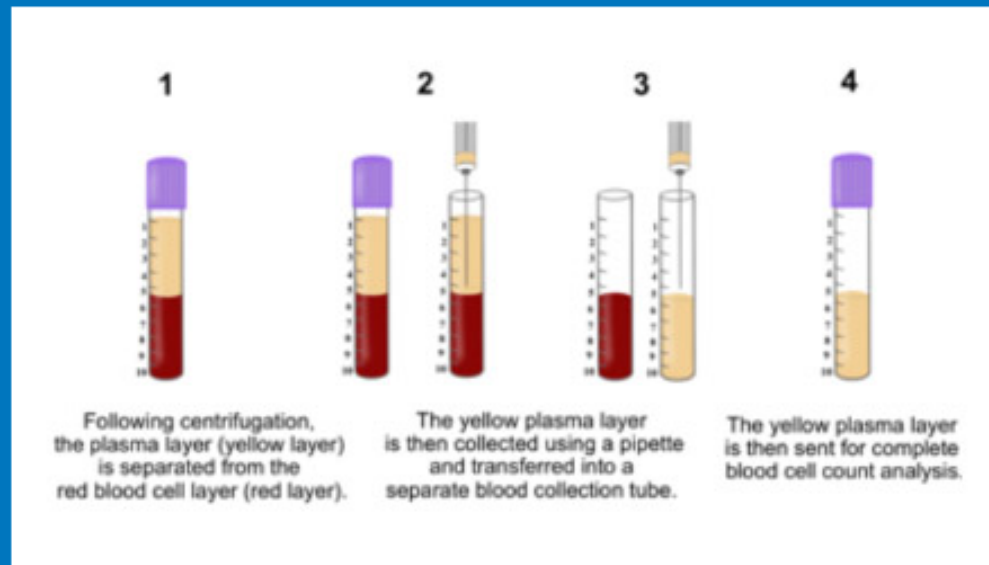


Figure: Commonly reported techniques for analyzing the effects of centrifugation on platelet concentrates.

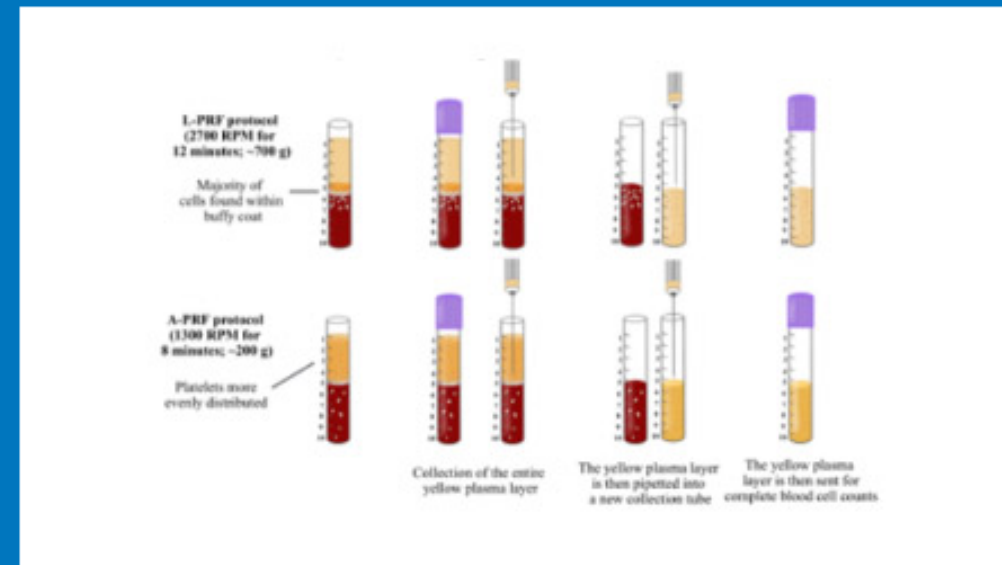


Figure: As a result, following centrifugation of both protocols, when both of these protocols are actually pipetting and sent for CBC analysis, only slight increases in cell concentrations is observed since the dense buffy coat layer produced using the L-PRF protocol is diluted with the rest of the sample. This does not reflect effectively the

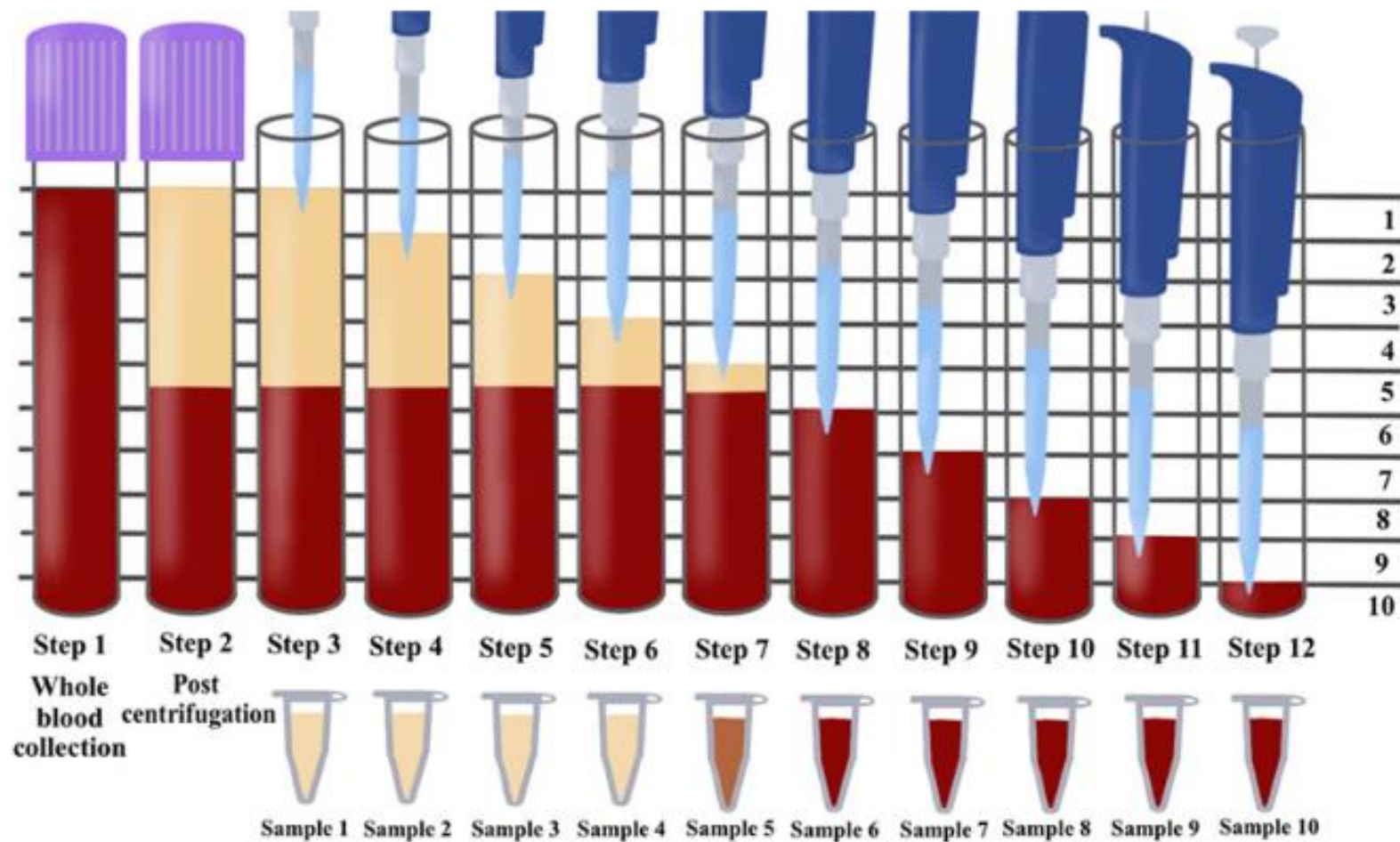
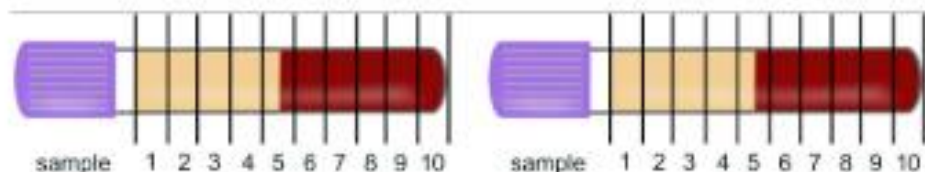
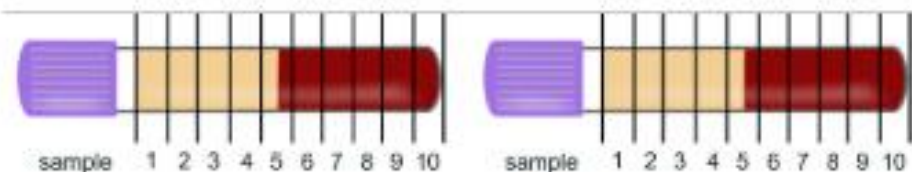
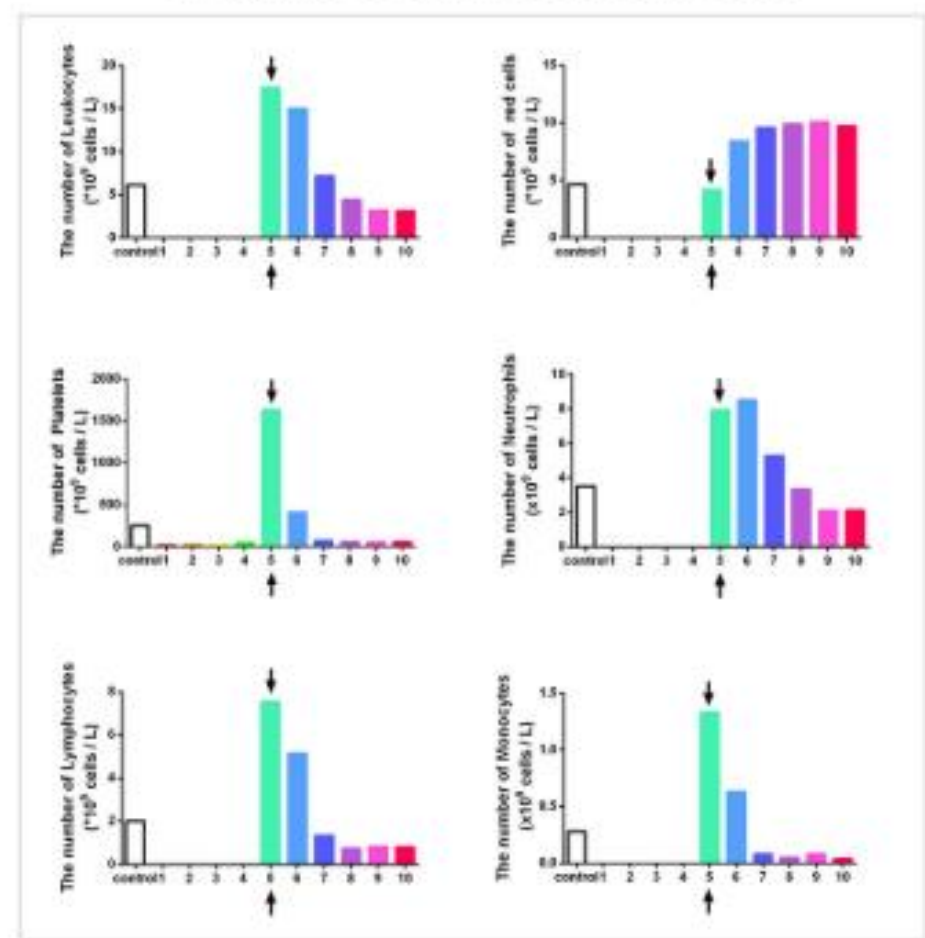


Figure: Illustration demonstrating the proposed novel method to quantify cell types following centrifugation of PRF. Currently, one of the limitations is that whole blood is compared to the total plasma concentration following centrifugation. This however, does not give a proper representation regarding the location of cells following centrifugation. By utilizing the proposed technique in this study by sequentially pipetting 1mL of volume from the top layer downwards, it is then possible to send each of the 10 samples for CBC analysis and accurately determine the precise location of each cell type following centrifugation at various protocols. Notice that one layer (in this case layer 5) will contain some yellow plasma and red blood cells. This is typically the location of the buffy coat where a higher concentration of platelets is typically located.

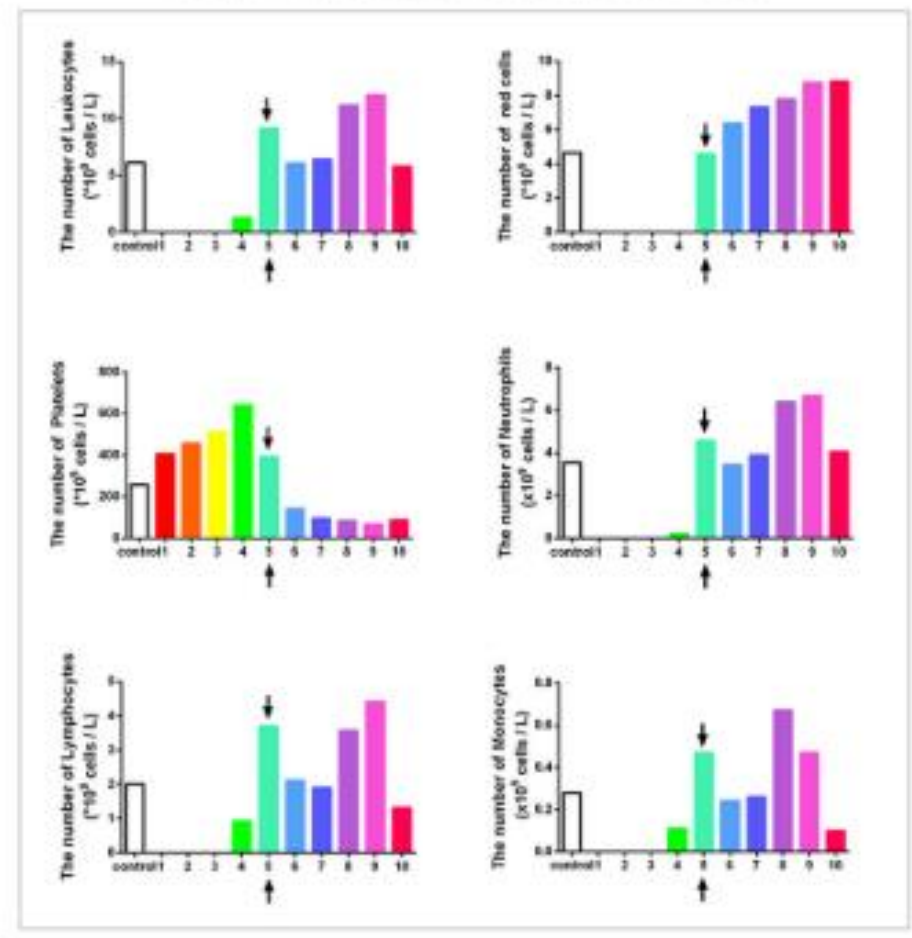
Comparing cell distribution in L-PRF and A-PRF protocols



Each blood tube as presented above is divided into 10 equal 1mL layers and quantified for cell numbers as presented below



Each blood tube as presented above is divided into 10 equal 1mL layers and quantified for cell numbers as presented below

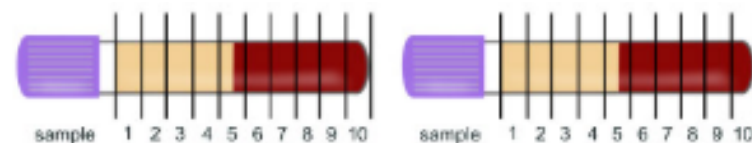


Horizontal Centrifugation: Bio-PRF (2019-present)

Very recently, a series of studies has shown that horizontal centrifugation of PRF is capable of producing significantly greater concentrations of platelets and leukocytes when compared to the currently available fixed-angle centrifugation devices most commonly utilized to produce L-PRF or A-PRF.

It was reported that one of the major disadvantages of fixed-angle centrifugation is that during the spin cycles, cells are typically driven along the back wall of centrifugation tubes at high g-forces. This exposes cells to high compressive forces against the back wall and must thereafter travel either up or down the inclined centrifugation tube based on cell density differences. Since red blood cells are larger and heavier than platelets and leukocytes, they typically travel downwards, whereas lighter platelets travel towards the top of the tube where PRF is collected. Noteworthy however, it was shown that on a fixed angle centrifuge, cells accumulate at the back walls of centrifugation tubes and the larger red blood cells entrap the smaller platelets/leukocytes below them and drag them into the red corpuscle layer. By utilizing a fixed-angle centrifuge, it is not possible to maximize platelet and leukocyte numbers as a result of fixed-angle centrifugation.

By utilizing a horizontal swing-out bucket centrifugation system (Bio-PRF), it becomes possible to fully separate cells and blood layers based on their density without necessitating cells to accumulate/damage on the back walls of centrifugation tubes. By utilizing horizontal centrifugation (Bio-PRF), it therefore becomes possible to isolate a higher number and concentration of platelets, leukocytes and monocytes when compared to either the L-PRF or A-PRF protocols.



Each blood tube as presented above is divided into 10 equal 1mL layers and quantified for cell numbers as presented below

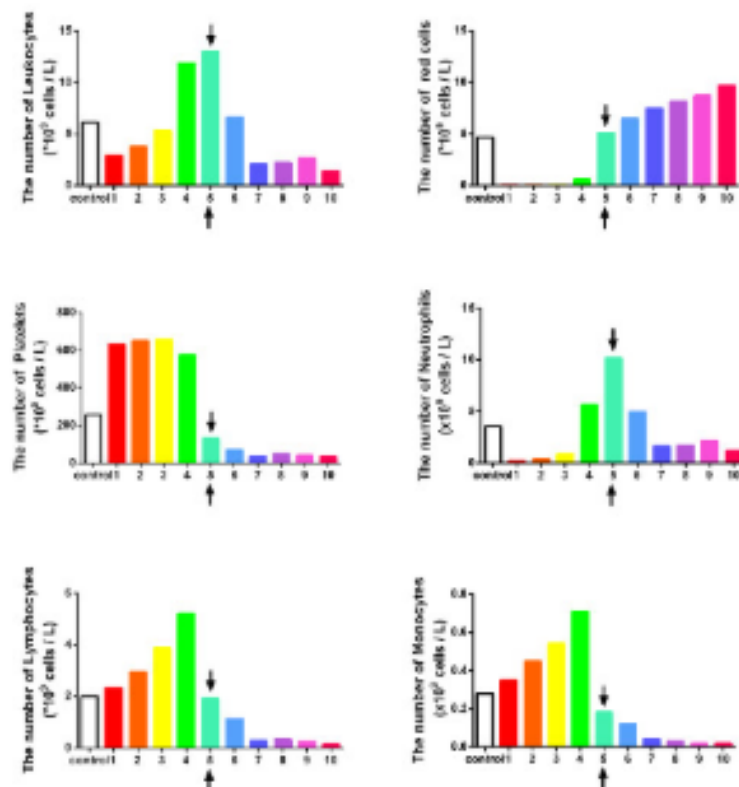


Figure: The concentration of cell types in each layer from 1mL down to the 10th mL sample utilizing the solid-PRF horizontal centrifugation protocol (700g for 8 minutes). Notice that most of the platelets as well as white blood cells are now more evenly distributed throughout the upper plasma layer. This is the first protocol generated for the production of PRF actually demonstrating an increase of white blood cells distributed throughout the upper PRF-layers.

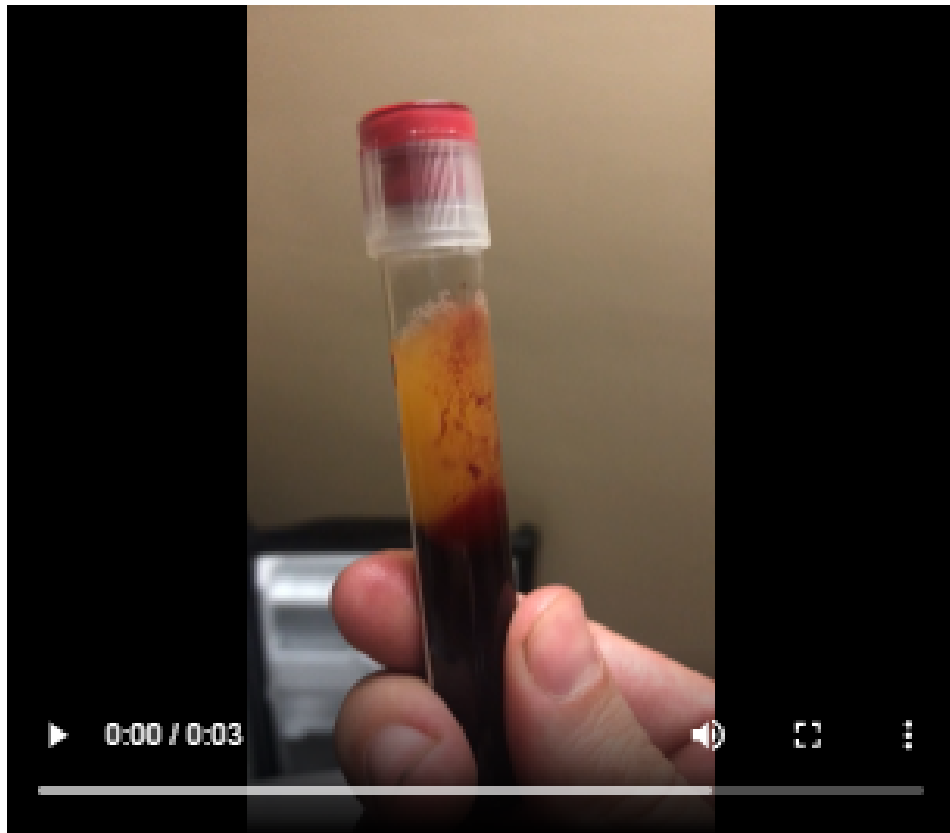


Figure: Owing to the large RCF-values (~200--700g), cells are pushed to maximize platelet and leukocyte numbers as a result of fixed-angle centrifugation. On a fixed-angle centrifuge, cells are pushed towards the back of centrifugation tubes and then downwards/upwards based on cell density. These g-forces produce additional shear stress on cells as they separate based on density along the back walls of centrifugation tubes.

In contrast, horizontal centrifugation allows for the free mobility of cells to separate into their appropriate layers based on density allowing for more optimal cell separation as well as less trauma/shear stress on cells.

By utilizing a horizontal swing-out bucket centrifugation system, it becomes possible to fully separate cells and blood layers based on their density without necessitating cells to accumulate/damage on the back walls of centrifugation tubes. By utilizing horizontal centrifugation, it therefore becomes possible to isolate a higher number and concentration of platelets, leukocytes and monocytes when compared to either the L-PRF or A-PRF protocols.