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FEATURE ARTICLE

Buffy Coats, Separation Gels, Centrifuging & Anticoagulants...

Why All PRP Systems Are Not the Same

BUFFY COATS, SEPARATION GELS, CENTRIFUGING & ANTICOAGULANTS...

Why PRP systems are not all the same

Platelet Rich Plasma (PRP) is an autologous therapy based on the release of growth factors from concentrated platelets derived from the patient's own blood.

Investigation into the use of PRP has been reported as early as the 1970s when it became clear that growth factors play a pivotal role in all types of wound healing. For just over 20 years, the application of autologous PRP has been safely used and documented in many fields including; orthopaedics, sports medicine, dentistry, ENT, neurosurgery, ophthalmology, urology, wound healing, cosmetic, cardiothoracic, and maxillofacial surgery. *(Sampson 2008)*

Since the start of the millennium, PRP therapy has joined the long list of medical treatments that cross over into the world of aesthetics. As the use of PRP as an injectable treatment has grown, regulatory bodies have become increasingly involved to ensure patient safety.

Harvest Technologies was one of the first PRP devices to gain U.S. FDA approval in 2000. There are now many PRP systems available globally which allow the clinician to create PRP from a small sample of a patient's blood in the clinic setting.

A non-exhaustive list of PRP system manufacturers and brands, available in the USA and/or Europe include:

- **Estar Medical - Eclipse®PRP, Cellenis®/ Tropocells®PRP**
- **Harvest Technologies - Harvest® PRP**
- **Arthrex - Arthrex Angel™ PRP, Arthrex ACP®**
- **BTI - Endoret® Platelet Rich Growth Factors (PRGF)**
- **Regen Lab - RegenKit®**
- **Arteriocyte Medical Systems - Magellan PRP machine**
- **Glofinn - Glo-PRP**
- **Centurion Scientific Ltd - PrO-PRP**
- **Dracula PRP**
- **Cascade Inc - Selphyl®**
- **Other systems are entering the UK marketplace, including products from Korea, Turkey, Italy, Spain and Greece.**

In the early 2000s, Estar Medical's PRP technology was one of the first to market in the U.K. for aesthetic indications, operating under the MyCells brand, a private label which was distributed by Kaylight. Prior to this, and to date, their original brand of Tropocells® PRP was globally distributed in parallel for medical applications only. Now, Estar's PRP system, sold in Europe, for aesthetic applications is branded as Cellenis®PRP.

In a report on the U.S. PRP market published by SmartTRAK, a Business Intelligence Hub for Life Science and Medical Device organisations in January 2016, they noted that:

"*...the U.S. aesthetics market segment with revenues of $6.6MM is expected to reach $9.3MM by 2019, with a CAGR of 8.9%. Facial rejuvenation applications represent 97.7% of this market and hair replacement procedures have the remaining 2.2% share.*"

Data for the U.K. is lacking but we do know that PRP is growing within aesthetics.

Regulation

Because PRP is re-injected into the body, the preparation system is generally recognised as a medical device by regulatory authorities. Each country's regulator decides on the class under which the medical device will be classified.

In Europe:

- To be used for re-injecting, a medical device needs to be CE Class IIb certified.
- IVD tubes used for separating blood during blood tests are Class IIa - not for re-injecting.
- There is no Class II on its own without a following sub class.

In China, Korea, Japan and the USA, the classification is equivalent to CE Class III. The clearance is per application and after submission of a clinical study.

All companies that produce devices that are Class II and III are subjected to an official audit annually and are subjected to unannounced audits by regulatory bodies.

IVD tubes are CE Class I certified and are for in-vitro diagnostic use only. No audit is required and it is a simple self-declaration from the manufacturer. Their intended use is not re-injecting into the body so systems with non-medical device compliant components should be avoided.

To avoid exposing your patients to unscrupulous, low cost, imported PRP devices, it is worthwhile noting also the following:

- CE Certificates are issued by Notified Bodies on official headed paper.
- A 'Declaration of Conformity' is self-declared and issued by a manufacturer. It is NOT a CE Certificate.
- A CE Certificate from a Notified Body is never printed on a manufacturer's watermarked paper or letterhead.

Range of PRP Systems

PRP preparation systems or devices that are available to the aesthetic marketplace today vary significantly in several key areas which we will explore further.

These are:

- **Composition of PRP**
- **Preparation methods (including centrifuging, buffy coats, gel separation and other approaches)**
- **Concentration of PRP**
- **Quantity of blood drawn**
- **Anticoagulants**
- **Activation protocols**

Composition of PRP

Nowhere in the definition of PRP is there a description for including red blood cells (RBCs) or granulocytes. These cells should be removed as much as possible from a PRP preparation. RBCs in particular are known to have inflammatory and catabolic effects – just the opposite of the desired effect of PRP.

Mononuclear cells from the while blood cell population are an important component of PRP however. They induce an anabolic effect, increasing collagen expression and fighting infection.[6]

If the prepared PRP in the syringe has any tinge of pink or red, it is most likely you are injecting a preparation that has RBC and granulocyte contamination. The ideal PRP solution will be a golden, straw-like colour.

- Haemoglobin is the protein molecule in red blood cells. The haemoglobin molecule contains iron which catalyses free radicals. These free radicals can induce apoptosis of host cells, in reaction to pro-inflammatory signalling. Because of these destructive capabilities, limiting red blood cell contamination in a PRP preparation is warranted.[1]
- In addition, Fredriksson et al, concluded that their results "*indicate that RBCs may participate in ECM homeostasis by inhibiting fibroblast proliferation and stimulating apoptosis*".[2]
- An excess of red blood cells triggers a pro-inflammatory response which in turn attracts more leukocytes [3] and can also exacerbate, rather than ameliorate, tissue damage.

To avoid an inflammatory response in excess of that caused by the injecting process, as well as apoptosis and inhibition of fibroblast migration, it is best to choose a PRP system that is not contaminated by RBC.

Preparation Methods

To eliminate risk to both the clinician and the patient, PRP preparation should be simplified with as few steps as possible. The number of parts required, including needles, should also be minimised. Risk is further increased if the clinician has to measure and add anticoagulant to the patient blood draw before centrifuging. The ratio of blood to anticoagulant is very important and precision accuracy is needed when preparing PRP. Drawing blood directly into the PRP tube, using a butterfly kit with holder, is also considered more effective than collecting blood into a syringe, with a needle, to then transfer it into a PRP tube. This extra step may result in infection and mistakes and takes up time.

Centrifuging

The majority of PRP systems use a centrifuge to separate the blood components.

Centrifugation depends on three inter-related features:

- Speed in RPM (Revolutions Per Minute)
- Radius of rotor arm
- Gravitational or G Force called RCF (Relative Centrifugal Force)

Please note that RPM is not the same as RCF. Therefore, be cautious when centrifuging a blood sample that requires a RCF of 1500G for example, that you do not set a speed of 1500 RPM.

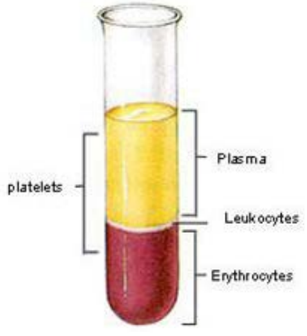
The difference between these two is further confused by different PRP systems setting different measurement parameters, and some do not even specify whether they are referring to speed (RPM) or to the gravitational force (RCF) being exerted by the centrifuge. Some basic centrifuges only have dials for speed (RPM) and time.

PRP Preparation Methods Fall Into Three Categories

1. Buffy coat - the oldest method of PRP preparation.
2. Advanced gel separation technology.
3. Machines which separate blood components using photospectrometry and fractionation.

1. Buffy Coat

This is the original method of blood separation and relies on the natural separation of blood components during centrifugation due to different densities. As shown in the diagram, a buffy coat is a "ring" formed between recognisable plasma at the top of the tube, the yellow part, and red blood cells below. Platelets are dispersed in and below this buffy coat "ring". Importantly, young platelets, representing the densest and the most active in their population, will be



forced to the bottom of the plasma. Attempts are then made to harvest platelets from this buffy coat ring while avoiding catabolic RBC and granulocytes contamination, with a resulting 20% lower platelet yield than might be expected[7].

Attempting to harvest younger, heavier platelets from the buffy coat means RBC and granulocyte contamination is considered inevitable. Buffy coat systems are sometimes known as "narrow neck" systems due to a tube narrowing design in the area of the buffy coat to assist with platelet harvest. Buffy coat systems often have to use two centrifuge spins usually at low speed.

2. Gel Separation Technology

Separation of blood using gel technology is an advanced method of PRP preparation, based on separating the different densities of blood components with the aid of a barrier gel. The technology is available in various forms from only a few companies, and was originally pioneered by Estar Medical technologies. Their gel is patented and proprietary, scientifically tested, and subject to major tests for patient safety submitted in studies to regulatory authorities. There is no evidence of patient risk from the use of the gel in over a million cases globally.

For the gel separation method, blood is collected directly into a vacuum tube containing a proprietary separation gel and anticoagulant. Following centrifuge, the aim is to separate the platelets from RBC and granulocytes, and elimate them as much as possible from the plasma via segregation behind the gel.

Essentially the gel enables a very much cleaner separation of plasma constituents from RBC than in a buffy coat method. How much platelet harvest, RBC, granulocytes and mononuclear cells are present in the plasma will vary, depending on the specific gel.

The platelets will be found on top of the gel. One such technology is further advanced with internal tube coatings to minimise platelet adherence.

Advantages of gel separation technology

- It generally results in high platelet harvest, upwards of 90%
- A single hard spin is considered sufficient to drive all catabolic RBC below the gel in most systems of this kind. Virtually all catabolic granulocytes are eliminated, leaving a desired mononuclear cell count from the white cell population.
- During the single hard spin, platelets are forced to the top of the gel and are simply re-suspended into the plasma by gently hand rotating the closed tube.
- Criticism is often levelled at the use of a hard spin by PRP systems who do not have gel separation technology. However, it has been demonstrated with platelet activation markers and growth factor release analysis which has been submitted to the FDA for approval, that high relative centrifugal force of 1500G does not cause cell activation or death in their PRP.
- The gel also enables adjustment of the plasma volume for PRP concentration and efficient resuspension of platelets within the plasma after centrifugation. This ensures maximum use of the high platelet harvest.

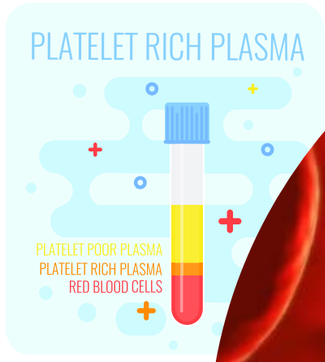
3. Machine Separation of Whole Blood

This is a high technology method of blood component separation using LED photospectrometry and fractionation. Such systems use the principle of light absorption by cells to separate the blood components. As there is no gel separation, this method arguably relies on a buffy coat separation principle, with a lower platelet harvest than is found in gel separation systems, and possible RBC and granulocyte contamination as found in buffy coat systems.

Concentration of PRP

Platelet Rich Plasma (PRP) is defined as a volume of the plasma fraction of autologous blood having a platelet concentration above baseline.[4] Normal platelet concentration is 200,000 platelets/μL.

After centrifuging with a gel separation system, increase in platelet concentration is 1.6x to 1.8x above baseline. Studies have shown that clinical efficacy can be expected with a minimum increase of 4× this baseline (1 million platelets/μL).[5]



PRP can be concentrated to higher levels by the simple process of removing Platelet Poor Plasma (PPP) directly after centrifuging. Analogous to removing solvent from a solution to increase the concentration of the solute.

A buffy coat system normally does not allow concentration of PRP. Some buffy coat systems attempt to facilitate an increased concentration of PRP with additional steps to the preparation process, and sometimes a second centrifuge cycle.

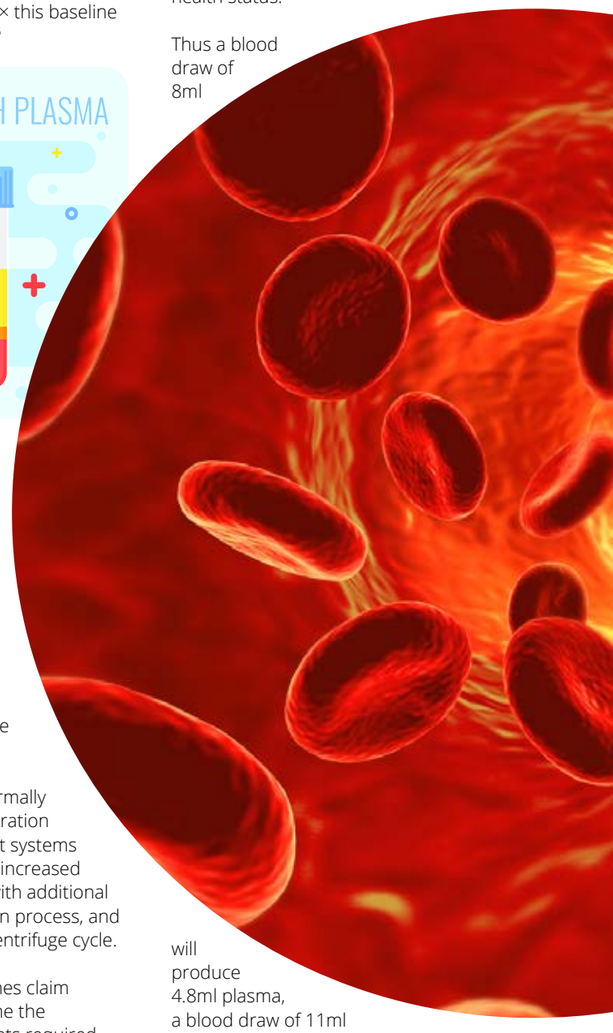
Some high tech machines claim the ability to programme the concentration of platelets required from the whole blood during the machine cycle. One gel system currently on the market allows easy and rapid concentration of PRP, with no RBC contamination, after a single centrifuge spin, and the removal of PPP.

Quantity of Blood Drawn

Average blood haematocrit will produce a ratio of plasma to RBC of 6:4. This will remain constant regardless of the volume or quantity of blood drawn, but will also depend on the individual patient's haematocrit, which can vary from person to person based on age, sex, health status.

Thus a blood draw of 8ml will produce 4.8ml plasma, a blood draw of 11ml will produce 6.6ml plasma, 20ml (12ml plasma), 22ml (13.2ml plasma) and 50ml (30ml plasma).

There will be a difference in the amount of plasma that is usable, without contamination with



"The ideal PRP solution will be a golden, straw-like colour..."

undesirable components, depending on whether a buffy coat system or a gel separation system is used. Systems using a buffy coat also typically have lower platelet yield and compensate for this with higher volumes of blood draw. With a gel system all the available plasma can be utilised, but depending on the specific system, contamination with RBC will vary slightly.

An important consideration for blood draw is whether the clinician wishes to concentrate the PRP before injecting. As we have discussed, concentration is achieved by removing Platelet Poor Plasma (PPP).

Amongst other factors mentioned above, a clinician will also choose blood volume draw depending on the indication being treated and how much concentration of PRP is required.

Anticoagulants

A number of different anticoagulants are used by different PRP preparation systems. These include:

- **NC** is sodium citrate with base pH about 8.5.
- **ACD** is citric dextrose anticoagulant with acidic pH of about 4.5.
- **MACD** is a modified ACD with a physiological pH of about 7.
- **NC7** is modified sodium citrate with a physiological pH about 7.

A base pH (higher than 7) or acidic pH (lower then 7) will cause stinging. A physiological neutral pH (of 7) will be more comfortable.

Anticoagulants used in many PRP systems are ACD solutions, with a pH about 4.5. To avoid stinging during injecting, some manufacturers and distributors of these PRP systems, are controversially recommending adding sodium bicarbonate to the PRP. Research into this 'DIY solution' for use in aesthetic application is unknown.

Aaron Esteron, CEO of Estar Medical, confirms that physiological pH PRP plays an important role in reducing platelet stickiness when injecting (compared to high or low pH that is available with regular ACD or NC) and that it also relates to better viability of platelets in a physiological environment.

Ideally, it could be said that you should be looking to invest in a system which uses a physiologically neutral anticoagulant, thus avoiding added pain for the patient, or the need to add bicarbonate of soda, as well as the advantages to platelet viability which is what we are looking for after all.

Activation Protocols

Growth Factors in PRP granules are released when platelets are activated. This activation can occur before injecting or occurs naturally in situ.

Activation of PRP before injecting

When PRP is activated ex-vivo before injecting, usually by adding calcium chloride or calcium gluconate, there is an immediate release of all growth factors. This activation also causes a thickening of the PRP to form a higher viscosity gel or even a clot (most commonly used for wound healing). Injecting needs to take place promptly.

In some countries, bovine thrombin is used to activate PRP; this is not permitted in Europe.

Non-activation of PRP before injecting

For optimal use of growth factors:

- growth factors should be released by platelets in-situ at the optimum time.
- releasing of growth factors ex-vivo, before injecting, may well reduce the PRP effectiveness compared with secreting of growth factors in-vivo.

Dr Allan Mishra, member of AAOS (Am Ass Ortho Surgeon) panel of experts reported that, "*Not all PRP is the same. PRP that has not been activated by thrombin or calcium may be the preferred form. The collagen within tendons can be expected to activate the platelets slowly, the preferred form. PRP activated by thrombin and or calcium, results in rapid discharge of growth factors, which may not be ideal.*"

In-situ growth factor release follows an efficient "just in time" concept. PRP systems that follow non-activation of platelets before injecting claim that optimal rupture of platelets in-situ, leads to more effective use of growth factors. PRP treatment after all, is a regenerative process taking place over time.

Activation of PRP in-situ occurs as follows:

1) Mechanical activation begins when inactivated PRP is injected via needle.
2) Activation continues during injecting when PRP meets with the patient's blood which contains thrombin.
3) Activation further continues when PRP meets collagen in the endothelial cell walls and extra cellular matrix (ECM). Collagen is a natural activator of PRP, thus when PRP is used in soft tissue, it does not need to be exogenously activated. *(Marlovits et al)*

Conclusion

There are some key points that you really should consider when investing in a quality PRP system for your clinic. It's very easy to be swayed by the marketing and glossy brochures as regulation of the marketplace is poor, and jargon can prevail when discussing the benefits of individual systems. My top tips would be as follows:

1. Always choose a PRP system which can demonstrate proper CE marking for a medical device, for re-injecting this should be a Class IIb.
2. Look for a high platelet harvest above 90% ± 10% for optimum release of growth factors.
3. Look for a system which requires no pre-activation before injecting the PRP.
4. Choose a system with a physiologically neutral pH anticoagulant, so that you are not thinking about adding sodium bicarbonate to neutralise acidity to avoid stinging upon injection.

5. You want no catabolic RBC [2,6] in your resultant PRP, with minimal catabolic granulocytes, whilst still retaining mononuclear cells from the white blood cell population.
6. You should have the ability to concentrate the PRP to at least the recommended 4x higher than baseline for clinical efficacy.
7. Finally, simplicity and ease of preparation should be a factor as it also minimises risk and increases safety for the patient.

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