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DEMYSTIFYING PLATELET RICH PLASMA

Mr Ansar Mahmood unravels the confusing subject of PRP Therapy

Using platelets in medicine has been around for quite some time, in fact, platelet lysates has been in use for over 40 years. Even in the cosmetic field, platelet therapies have been in use for dental and maxillofacial surgical applications, as well as wound healing, for around 30 years. However, only approximately 10% of platelet lysate use is in the clinical medicine arena. The other 90% is in research and transfusion laboratories for cell studies. Scientists have discovered that it is a perfect environment to enrich, grow, and keep cells for study alive for longer, such as when studying stem cells, compared to putting them in an artificial growth substrate.

When it comes to the medical use of platelets, it seems to cycle through the medical literature, but sadly, has suffered from a common phenomenon in medicine, depicted in Scott's Parabola. This is where there is an innovation in medicine or surgery, innovators and expert opinion leaders pick it up and run with it, they get good results which they publish in a journal, and the wider medical fraternity hears about it and jumps on the bandwagon to start using it. It is at this point that practice diverges, understanding and knowledge of protocols and how to use it dilutes from the original expert users, and clinicians lose faith because their results are very variable/non-reproducible. It then slowly disappears and may resurface after many years. This is often mentioned in conjunction with failed treatments and specifically surgical procedures.

My firm view is that PRP has now overcome this phenomenon and is here to stay. However, risk remains, and this is almost entirely to do with clinicians' lack of understanding of the properties of plasma-based therapies and how to translate them into clinical practice. The biggest worry to me when speaking to clinicians, and even scientists occasionally, is that there is a dangerous assumption that all PRPs are equal, and all indications are equal, but there is a huge variation between them.

If you intend to use PRP in multiple fields such as dentistry, wound healing and burns, orthopaedics, or aesthetic rejuvenation of the skin or hair, then you must consider that all the environments you are dealing with are unique. The blood supply in those areas, the stem cells you want to influence, the growth factors – for some you need to boost fibroblast activity, for others, it is keratinocytes, chondrocytes or tenocytes - they are different cell lines. Each of these cells which you are asking the PRP to act upon have a different nutrient requirement and are in variable oxygen tension environments.

Therefore, you cannot assume that one PRP is going to do the same thing everywhere that you place it. I find that this is the number one reason why practitioners lose faith in PRP because they lack extended knowledge and have just one PRP in their arsenal which they try and use everywhere, achieving good results in one area, but poor results everywhere else, which they go on to blame on the PRP. It is not the PRP that is the issue, the issue is using something that is not fit for purpose or needs to be manipulated in a certain way to make it work in a specific area, or in a more hostile environment. The 'failure' is not in the tool but rather the clinicians' understanding of how to utilise and manipulate the tool to achieve the best outcome.

What is PRP?

PRP is a fraction of plasma from the blood which concentrates platelets, hence the name is very descriptive - platelet RICH plasma.

There is, however, no universal definition for the rich part of PRP because it is bespoke to the individual. One person's baseline (full blood) platelet count will vary greatly to that of another person, up to two or threefold, whilst being completely normal for each individual.

For example, one patient may have a normal baseline platelet count in serum of 150,000 per microlitre, whilst another is 300k, and another of 450k may be normal. Attempting to define a good, generic platelet concentration level for PRP would be difficult when every patient's baseline level is unique to them, and you could simply be giving one person what they already have, with no real concentration or enrichment in platelets if you chose an arbitrary figure based on the lower end of normal. Similarly, as mentioned previously, you are dealing with different tissue environments when treating skin or hair, for example, thus in one environment a one and a half-fold concentration may be adequate, but in another, you might need an increased dose, such as four or five-fold concentration and occasionally even higher. I do believe a minimum standard is required but may need to be indication specific.

PRP systems

The original systems used for PRP were simple. If you take a sample of someone's blood in a tube and spin it in a centrifuge, once you hit a certain speed, you start to get separation, based on the density of the components. The red blood cells tend to be iron- or haema-rich and are heavy, so they drop to the bottom of the tube. The leukocytes, the white cell layer, is the next layer in the tube as it is a bit heavier than the plasma and platelet layer, generally, although, large platelets and small leukocytes can be of similar density. These leukocytes sit in the middle and are referred to as the buffy coat. Remember, this is using a plain tube, without any of the gels or anticoagulants which came later to assist with mechanical separation, this is simply separation by centrifugation.

Buffy coat systems were the original PRP systems and are still on the market today. This early process is no different to how laboratories process blood samples and analyse the separate component levels in patients in daily clinical practice.

However, when you are harvesting platelets from a buffy coat system, you can contaminate your PRP very easily with the red blood cells or leukocytes, which may not be desirable. The red blood cells sit just below your plasma, but if you do not go down far enough into the plasma you will not pick up the platelets which are sitting on top of the very thin buffy coat, which can be a fraction of a millimetre in width depending on the type of centrifugation and volume collected. Platelets themselves are exceedingly small, between 2-5 microns in size, hence, even viewing through an electron microscope you can struggle to see them individually unless they are stained. With soft spin systems like injectable platelet rich fibrin (iPRF by Choukron) or Autologous Conditioned Plasma (ACP by Arthrex), you cannot differentiate the buffy coat from the rest of the system. Your choice is often to either accept contamination with red blood cells and catabolic leukocytes that can cause inflammation or accept a reduction in the number of harvested platelets by conservative extraction. This often means these systems require a steeper learning curve and hence inevitably more variability in the end-product being injected, particularly when first starting out.

Later, companies involved in the research and development of PRP realised that certain tube types, whether made from glass or plastic, have a certain density and the material has a certain 'stickiness' which can be modified by adding coatings to the inside of the tubes. Next, they researched the effects of the centrifuge speeds, with certain volumes of fluid (blood), in different tube sizes with certain coatings. This is where the difference is seen between the competing companies, the R&D studies to maximise the platelet count in your tube and purity, as compared to simple buffy coat systems.

Not content with producing concentrations of platelets which may be in some systems as low as 1.5 -2 fold above the baseline patients' count, many practitioners have looked to maximise platelet concentrations to fourfold, eightfold, or tenfold, in the belief that more is better. One way to increase your concentration is to take a larger volume of blood in the first instance, or you could double or triple spin your sample to extract as many platelets as possible. For example, if you are using a buffy coat system, you spin it once and end up with between three and eight millilitres (mls) of plasma at the top of your tube. This may be double or triple the baseline platelet concentration but rarely any more than this. You could then extract that plasma, put it into a separate tube, and run the centrifuge again so you have performed a double spin which concentrates it down again and most of your platelets will be in the bottom half of your tube, giving you approximately half the volume ready to inject. This double spin often turns your two or threefold concentration into a six- or eightfold concentration. However, that is all quite laborious, and requires a knowledge and understanding beyond most practitioners. Similarly, by extracting the plasma to a new tube to achieve the double spin you may break sterility, which is far from ideal where some treatments may require a closed system for safety from infection.

Where there is a problem, there is usually a solution, and the manufacturers of PRP systems started to come up with the use of thixotropic gels, aiming to make things simpler and more reliable. There are now multiple gel-based PRP systems on the market. Under high G-force in the centrifuge, the thixotropic gels have an interesting mechanical property, in that they become liquid. During the spin, the gel moves up the tube and based on a fixed volume of blood in a specifically designed tube, spun at a pre-set speed for that system, the gel will slide to just the right point to create a mechanical barrier between the red blood cells, the buffy coat, and the plasma. These tubes also contain an anticoagulant. Due to the solid mechanical barrier, you can tip the sample without any risk of red cell contamination, so they are ergonomically advantageous. Another distinct advantage is the 'one step' platelet concentration process, for example, if you started with 12mls of blood and you are left with 6mls of plasma above the gel. You can remove and discard as much of the top portion of the platelet poor plasma (PPP), in the knowledge that you will have a platelet rich portion in the bottom 1-2mls which is sitting on top of the gel. Although, you will need to resuspend it, by rocking or gently shaking the tube, to disperse the platelets in your selected residual volume, as the high G-forces in the centrifuge tend to make them clump together at the gel's surface and around the glass adjacent to the gel. Assuming you can get close to 100% (for ease of maths) of your platelets from the whole blood into your plasma and you start with 12mls of blood and now have all the platelet yield in 2mls of plasma your concentration has increased sixfold. In reality, no system yields 100%, I have

seen this vary between 30% - 85% for various common systems we have tested in the market.

Gel-based systems are regarded as very efficient at providing a 'pure' PRP, at a high concentration after removing the platelet poor fraction. Plus, you can harvest the PRP without the risk of contamination or platelet loss that you would incur with a buffy coat system. However, with significant amounts of money needing to be spent on research and development, safety testing of the gels and anticoagulants, plus applications for CE marking or FDA approvals, we see ever more complex systems appearing which can create confusion for practitioners, and inevitably these systems can be more expensive than the original buffy coat options. Many people simply do not know what to invest in, especially when they see cheaper buffy coat systems still being actively marketed, some of which use cheaply made intravenous diagnostic (IVD) tubes with no patient safety testing mandated on these types of tubes as their product was never intended to be used in patients. As a result, they often lack the class IIb CE marking (soon to be class III under the MDR if they contain anticoagulant or other additives) of the established buffy coat systems or many of the gel-based options. Nomenclature can be very confusing for clinicians (see table).

It is worth noting that there are very few studies comparing PRP systems in clinical practice and showing valid recording of clinical outcomes. Virtually all the stated comparative benefits of a system are based on in-vitro (laboratory) data. As a researcher and clinical practitioner involved in the Institute of Translational Medicine, I know that the behaviour of cells and platelets in a lab versus a living host are not comparable. We all know the relatively evidence poor zone that aesthetic medicine inhabits, however, I rarely see in any field of medicine or surgery the frequency of spurious claims in this market by manufacturers and distributors, which simply do not stand up to even basic scrutiny.

There are multiple PRP based products that due to mild variations in preparation are sold as the 'next big thing' or a 'new generation' of PRP. A recent example I have seen gaining traction is iPRF which is marketed as 2nd generation PRP or even 'advanced' PRP. To my knowledge based on a current literature search there is no validated, peer-reviewed, comparative study justifying any of the claims made about iPRF outside of a laboratory. Any manufacturer or distributor may make a case that their product works in a given clinical indication, but to claim superiority there is a well-established route of conducting a non-inferiority trial and this does not exist for iPRF; in the absence of this they are at best unsubstantiated claims and at worst lies.

By stating that the lack of an anticoagulant means that iPRF behaves differently is technically incorrect, because the common anticoagulants used in PRP systems are reversed within seconds to minutes on injection by normal endogenous calcium haemostasis in any patient. Some systems also mandate activation for PRP on injection, ensuring rapid and early release of growth factors in the area of injection which would also generate the fibrin mesh and matrix rapidly within seconds to minutes after injection, so in theory they also behave comparably to iPRF. Moreover, the principles of preparation of iPRF, which are sold as 'new', is incorrect, this method of preparation has been around and known about for over 10 years. More well-designed studies are needed in this area. Unfortunately, like anything in the consumer and B2B market, *caveat emptor* applies, and there are far too many clinicians that are willing to part with their money without due diligence and asking the correct questions.

Name	Separation Type	Reality
PRP	Gel or Buffy available	All of these are PRPs or derivatives of PRP with minor variations. Their variation is due to the R&D processes and in most cases the commercial and marketing drivers of the individual companies to differentiate their products. That is not to say they are in anyway equivocal in their output of the end-product and growth factors.
PRGF - BTI	Buffy	
iPRF – Choukron/ Smartcell	Buffy	
L-PRP	Gel or Buffy available	
LR-PRP	Buffy	
A-PRP	Buffy	
ACP	Buffy	
PRGFM	Buffy	

Concentrations

The temptation is to think, more, more, more platelets... if something is good or works, the more of it, the better. However, this is not always the case in medicine. Research on skin has shown that, like all biological systems, your body only has a certain capacity to take biological feedback before it saturates and there are mechanisms in place to prevent overload. If you go beyond a certain threshold of PRP concentration, certain growth factors will stop functioning optimally. Studies have demonstrated that if you place platelet concentrations beyond five- to eightfold into the skin - some of the PRP systems can produce twentyfold concentrations – then you will not only not notice any incremental benefit, but in fact that you see a plateauing of the cellular response up to about eightfold concentration. Any higher concentrations beyond that have in fact been shown to create a reduction in response by about 10% lower than if you had used the lower concentration.

Uses for PRP

PRP should be regarded as a biological normaliser. When we are young, we usually have wonderful skin which is rich in hyaluronic acid and stem cells, because fundamentally the skin is behaving normally with minimal effects of ageing or environmental factors.

If skin behaves normally, then its balance is right, its protection is right, it hydrates itself, and it repairs itself. It is only when any of those mechanisms are out of balance that you start to see damage outside of normal ageing.

PRP can generate an injury response in your body when it is placed in the skin or any given area and fundamentally sets up the start of a signalling cascade which causes the recruitment of cells to heal and repair the area. Senescent stem cells will be woken up, growth factors will be rallied, fibroblasts, transforming proteins, and cytokines

will be enlisted to help form new cells in this area, to bring the integrity of that tissue back to a 'normal' phase. PRP is not designed to make a 'super you', but a 'normal you', which is what we want as good aesthetic practitioners. We accept your 'normal you' may be better than your 'current you'! Patients have problems with their skin because it is not behaving in the normal way that it should be, PRP can optimise and maintain normal. It will also stop free radical damage, stop the activity of inflammatory cytokines, making it a potent anti-inflammatory, which is why it helps in arthritis.

Clinical effects

Data and studies are showing increases in skin density from PRP treatment, one of the few treatments that does this directly and naturally. There is an increase in fibroblast proliferation in the skin, which is increasing elasticity. Similarly, anything that is behaving abnormally, like melanocytes, for instance, creating sunspots or uneven skin tone, can be brought back to normal by the actions of the PRP. It normalises the function of the melanocytes resulting in a more even skin tone. PRP is also anti-inflammatory, so it reduces the damage from cytokines and free radicals in the area treated. Overall, it helps you to maintain skin at a better level, and the beauty of it is that it is natural.

Managing patient expectations is key and that begins with a good medical consultation. Be honest with your patients about the results you think you can achieve, the data that is out there, and what you hope they will gain from it. There is a lack of good, randomised, controlled trials for many of the indications for PRP, but not an absence, so know your literature and how to analyse the better-quality studies. We are all guilty of reading abstracts rather than the body of a paper but given the variation in preparation and PRP systems it is especially important we scrutinise the methods, in terms of quality of PRP, protocols, concentration or number of treatment sessions, which may vary significantly between indications and systems. As a practitioner, you need to decide what you believe and what you practise, based on the evidence available to you. If you demonstrate what you know and admit what you do not, that builds trust and patients always respond positively to that even if they decide not to proceed with treatment!

Qestions to ask

If you are considering adding PRP treatment to your clinic, there are some basic questions that you should ask any medical device manufacturer or distributor who is trying to sell you their system:

- **Safety data and CE mark verification from the notifying body?** Any manufacturer that has achieved a valid one should be willing to share this with you immediately as a badge of honour for their system as they are not easy to obtain and can be validated with the notifying body's website. If you check the notifying body's website and they are not on there, be wary.
- **Any white papers and research data specifically on their system?** Any respectable manufacturer will have this, but many hide behind the data of other manufacturers by stating they are the same or similar. Even if it is an OEM product, the new MDR regulations state they must have the complete data file on record, not simply trust that whoever made the tubes or system for them knew what they were doing, and they have just crossed their fingers and hoped for the best! (Remember the PIP breast implant scandal.)
- **What is the yield or efficiency of the system?** Simply this is asking how many of the platelets from the whole blood are delivered for you to use in the plasma.
- **Can you vary the concentration, should you choose to, and is that easy to do?**
- **Can you select to have leucocyte and/or monocytes in or out of the plasma?** In the aesthetic sphere monocytes are increasingly recognised as valuable, however, other leucocytes are rarely beneficial. But in some orthopaedic indications, leucocyte-rich may help.
- **Does it eliminate red cell contamination?** This is rarely desirable whether treating skin, hair, joints, or tendon.
- **Is it easy to use and produces a reliable PRP with a low failure rate?** Patients generally do not like being stuck with a needle for a second or third time!
- **If it fails, is there an easy rescue without repeating the whole process?** Tips and tricks for most systems can be gleaned from good training providers and hopefully avoids you rebleeding your patient or wasting a tube (and money).
- **What support is available?** Support capability of a manufacturer is a broad topic and extends beyond the sales rep, do they have a clinical advisor or expert that can offer clinical advice and protocols etc?
- **Cost?** I have intentionally placed this as the final question as it should only be asked if all the above questions have been adequately answered and then cost may become the final consideration. I have experience of many systems and would never sacrifice patient safety or my outcomes for the sake of £20-40 which is often the maximum price difference between the reputable systems.

The future

The future of PRP will be in the study of growth factors and their activity in relation to progenitor cell action. Researchers are producing bespoke delivery methods and customised PRPs. I believe that we will, at some point, end up with PRPs or derivatives that are specifically for hair, joints, skin, and wounds etc. It may not be a PRP as we know it, perhaps a proteomic solution that has a certain quantity of platelets within it and is rich in specified growth factors.

Regenerative medicine is the future of all medical disciplines including aesthetics and anti-ageing, and I feel privileged to be contributing to research and developments in this area.