Comparative Study of Platelet Count: Injectable Platelet Rich Fibrin (i-PRF) Compared to Platelet Rich Plasma (PRP). -Controlled in vitro laboratory study

Experiment Findings · March 2020

DOI: 10.13140/RG.2.2.17779.89125

CITATIONS
0

READS
23

2 authors:

Tania Malan
Uniskin Wellness Clinic
2 PUBLICATIONS **0** CITATIONS
SEE PROFILE

Anita Woolley
Sheffield Teaching Hospitals NHS Foundation Trust
15 PUBLICATIONS **165** CITATIONS
SEE PROFILE

Comparative Study of Platelet Count:

Injectable Platelet Rich Fibrin (i-PRF) Compared to Platelet Rich Plasma (PRP).

- Controlled in vitro laboratory study.
- Department of Haematology, Royal Hallamshire Hospital. UK

Tania Malan
INP, MSc Aesthetic Medicine

Anita Woolley – FIBMS
Senior Biomedical Scientist

Purpose:

To determine the composition of PRP obtained from 4 commercial separation kits, which would allow assessment of current classification systems used in cross-evaluation comparisons.

Introduction:

In the last few decades different concepts have evolved for the best possible tissue regeneration in wounds, surgery and skin (Choukroun 2017, Dohle 2018). The use of PRP and its growth factors is attracting much attention amongst aestheticians, dermatologists and surgeons (Takura, 1996, Yildiz, 2016, Wang, 2016). Platelets contain high quantities of growth factors capable of stimulating cell proliferation (mitogenesis), matrix remodelling (Dohan, 2009) and vascular growth (angiogenesis) (Robert, 2001). See Table 1.

PRP is a relatively new biotechnology which is part of tissue engineering and cellular therapy (Robert, 2001). PRP originated 40 years ago where blood derived products were used to seal wounds and stimulate healing (Dohan, 2009). Some studies found that the drawback from PRP is that the additional use of anticoagulants inhibit wound healing (Wang, 2016). Furthermore, the optimal combination of each cellular component in PRP remains unknown due to the variety of commercially available PRP generating systems, which makes it difficult to know which PRP preparation is best for which clinical indication (Boswell, 2014).

Now a second-generation protocol emerged known as PRF (platelet rich fibrin) or Choukroun's i-PRF. It differs from PRP in the sense that there is no anticoagulants or additives and are centrifuged at lower speed (Dohan, 2006). PRF has a mixture of platelets, white blood cells, bone morphogenic protein (BMP), stem cells and growth factors (Holistic, 2018). However, these concepts have spurred commercial interest and exploitation with a wide variety of preparation protocols and kits (Dohan, 2009).

More confusing is the various nomenclature depending on who markets the product such as Vivostat PRF, Fibrinet Platelet Rich Fibrin Matrix (PRFM), Dracula Kit etc. Various classifications also exist such as L-PRP i-PRF, PRF etc. See Table 2. The use of platelet concentrates, and its efficiency is controversial (Dohan, 2009). Dohan (2009) continues that applications and methods leads to a different product with different biology and potential uses. A literature review of the various classifications and impact added to the confusion as the protocols, machines, activation methods are all different. Furthermore, the literature mention "large" or "rich" concentrates but no real evidence of its numbers could be found within the literature. Therefore, studies are mostly empirical with no real data behind it and no standardisation. The literature remains unclear about the true clinical differences between these related fibrin products (Dohan, 2008). Dohan (2008) continues that the differences between PRP and i-PRF have never been analysed with regards to its leukocyte content or the final concentrate key parameter of platelets and never been accurately documented. The problem is that there is only empirical data available regarding the various centrifuges, protocols and platelet yield (Redaelli, 2010). The tests are done by "eye" where clinicians or commercial sellers judge the number of platelets which often based on the amount of plasma separated from the red blood cells. The aim of this evaluation was to determine the cell count of 4 commercial kits to accurately measure the difference cell counts, but most importantly the amount of platelet concentration in each kit.

Methodology:

Four normal adults each donated a total of 141 mL of whole blood, some of which served as a control and the remainder of which was processed through three different PRP and one i-PRF separation kits. The resultant PRP was tested for platelet count, red blood cell count, and white blood cell count, including differential in a commercial pathology laboratory. Quantitative research seemed to be the best approach with the aim to use computational techniques to extrapolate numerical data accurately and then compare the numerical results. Therefore, a comparative analysis will be undertaken to determine:

a) The baseline of a normal cell platelet counts within blood without being centrifuged – control sample

b) The platelet counts (concentration) of each kit after they have been centrifuged according to their manufacture protocols.

c) Evaluate the cell count in sample tubes containing sodium citrate anticoagulant and gel separator compared to sample tubes with no additive or gel separator

d) Compare the protocols and their impact on the platelet and cell concentration.

e) Attempt to provide a quality parameter in platelets within each type of protocol.

The purpose of comparative analysis is to compare what has happened, or what is likely to happen in the absence or presence of certain features (Smelser, 1976). The starting point of comparative analysis is the explanation of similarities and differences and to explain the differences (Pickvance, 2005) or its

cause and effect (Bukhari, 2011). According to Lancaster (1997) the goal of comparative analyses should be the assessment of rival explanations. Comparative study is used to determine and quantify relationships between two or more variables by observing different groups which also includes different protocols or treatments (Bukhari, 2011). Bukhari (2011) continues that comparison focuses on a few specific characteristics. In this study the focus is on number of platelets available in plasma after being centrifuged. As well as the difference in platelet outcome after centrifugation following different protocols and the additives within. Another aspect of comparative study is the role it plays in concept formation by bringing into focus suggestive similarities and contrasts amongst samples (Bukhari, 2011). Bukhari (2011) continues that if differences are well explained it can strengthen our position through research and primary arguments.

The type of comparison classification started off with Individualising comparison where a small number of samples are evaluated on their platelet production following their protocol (Tilly, 1984). Individualising comparison involves discovering how different cases are, such as difference in platelet yield in this case (Pickvance, 2005). A second type of comparison study is then undertaken to explore universal comparison with the aim to establish that if the same rule is followed if the platelet outcomes will improve (Tilly, 1984). Therefore, would platelet counts be higher if spun at low speed?

Materials and Methods:

Laboratories use automated analysers to provide clinical and accurate interpretation of blood specimens. Therefore, in this comparative analysis the Department of Haematology, Royal Hallamshire Hospital, as a UKAS accredited centre conducted a comparative analysis of PRP and i-PRF in three separate tests over a three-month period involving multiple male and female donors.

I. EDTA sample was taken from each donor to measure the baseline.

II. Blood samples were taken using each commercial tube.

III. Blood samples were spun using the Smart Cell centrifuge, according to manufactures protocol.

IV. 2ml of platelet concentrate from each tube was harvested according to manufacturer's instruction and transferred to 75x12 polyproline tubes using a calibrated Proline Plus auto pipette. Each polyproline tube was labelled with a bar code prior to being tested.

V. An independent haematology biomedical scientist tested each sample using the Sysmex Analyser XN2000.

Protocol:

Protocol						
	Time (minutes)	**Speed (RCF)**	**Supernatant extracted (PPP*)**	**Volume Tested**	**Anticoagulant**	**Gel Separator**
Baseline	NA	NA	NA	5ml	EDTA	No
PRP A- 11ml	10	1423	4.8ml (80%)	2ml	Sodium Citrate	Yes
PRP B- 10ml	5	1500	3ml (50%)	2ml	Sodium Citrate	Yes
PRP C – 10ml	5	1500	3ml (50%)	2ml	Sodium Citrate	Yes
i-PRF – 12ml	7	66	0	2ml	No Additive	No

**PPP = Platelet Poor Plasma*

Results:

Means Cell Count					
Cell Count	**Baseline**	**i-PRF**	**PRP A**	**PRP B**	**PRP C**
Platelet [10^9/L)	290	540	179	125	243
White Blood Cell [10^9/L)	6.61	4.95	1.14	0.84	0.54
Red Blood Cell [10^12/L)	4.48	0.06	0.01	0.01	0.02
Platelet % (-/+)	NA	+86%	-38%	-57%	-16%

Leukocytes					
Cell Count [10^9/L)	**Baseline**	**i-PRF**	**PRP A**	**PRP B**	**PRP C**
Monocytes	0.54	0.67	0.24	0.15	0.08
Lymphocytes	2.16	3.84	0.88	0.68	0.45
Neutrophils	3.74	1.03	0.13	0.01	0.01

Summary:

The PRP results show a platelet concentration significantly lower than the baseline. i-PRF results shows a much higher percentage above the baseline. Furthermore, the leukocyte count, although higher in the i-PRF results, are still lower than the baseline. The relevance of this are discussed later in paper. The results raise several questions which will need further exploring. Does PRP not work in some cases because there are minimal platelets and that is why some studies have mediocre findings? Are we sold

snake oil by large organisations who only have a commercial interest? Are the results from PRP due to the micro injury caused by micro-needling to administer the PRP or is there a PRP protocol that yield better platelet counts but just haven't been measured? The i-PRF results seems to be much more promising in comparison to PRP. The low speed centrifugation concept (LSCC) indicates that higher number of leukocytes and platelets can be obtained by reducing the relevant centrifugation force (RCF).

Discussion:

Why the interest in platelets?

Platelet activation plays a key role in the body's natural healing process. The role of platelets in healing especially the coagulation cascade has been well documented (Stellos, 2010). What we know of platelets is that it plays a key role in maintaining blood vessel wall integrity by adhering to defects within the vascular wall, by sealing the lesion and initiating the healing process (Stellos, 2010). The influx of platelets is an early event in the process of wound healing and contributes to signals which are critical for tissue regeneration (Reese, 2010). A collagen receptor (α2β1 and GPV1) stabilises platelets to the exposed sub endothelium (Anitua, 2004) and activates collagen fragments, thromboxane A2, adenosine diphosphate (ADP), as well as growth factors, cytokines and chemokines (Abrams, 2001). Platelets have a complex storage system in the form of intracellular granules that allow them to transport many biologically active molecules. They also contain a series of antibacterial proteins called thrombocidines. The bioactive molecules promote tissue repair and influence the reactivity of vascular and other blood cells in angiogenesis and inflammation (Anitua, 2004). Anitua (2004) continues that platelets contain storage pools of growth factors (see table 1). More than 800 proteins are secreted in the platelet matrix (Kawazoe, 2012).

The fact that platelets can secrete growth factors and active metabolites means that their applied use can positively influence rapid healing and tissue regeneration (Anitua, 2004). Anitua (2004) explains that newly released or exposed proteins and other substances stimulate tissue repair and vascular remodelling. The extracellular components (glycosaminoglycans, collagen and adhesive proteins) bind growth factors and establish a chemotactic gradient (increases chemical concentration) for cell recruitment, including a storage pool within the matrix (Anitua, 2004). Platelets contain a broad arsenal of active substances such as cytokines (growth factors), chemokine (signalling proteins) which can be modulated into tissue regeneration through paracrine fashion (cell to cell signalling) (Stellos, 2010). Stellos (2010) further explains that by activating platelets it triggers substances (α-granules, dense granules and lysosomes) within the platelets which contain bioactive mediators which secrete the following factors tabled below. Therefore, platelet derived growth factors can influence cellular growth, morphogenesis and differentiation to accelerate healing (Anitua, 2004). The role of fibrinogen (Fg) is as an adhesive protein alongside fibronectin (Fn), vitronectin (Vn) and thrombospondin – 1 (TSP-1). Fibrinogen is an abundant and a proportion becomes attached to platelet receptors and participate in thrombus growth and acts as a mitogen (cell mitosis) (Anitua, 2004).

Table 1: **Growth factors and their actions** (Steed 1997, Stellos 2010).

Growth Factors in PRP	Actions of Growth Factors
Platelet derived growth factor aa (PDGFaa), (PDGFbb), (PDGFab), (PDGF)	Chemotactic (movement or orientation of an organism of cell along a chemical concentration) for fibroblasts and macrophages. Mitogenic (cell division) for fibroblasts, smooth muscle cells and endothelial cells. Powerful chemoattractant and stimulator of cell proliferation.
Transforming growth factor beta-1 (TGF-b1) (TGF-b2)	Mediates angiogenesis Chemotactic for fibroblasts, keratinocytes and macrophages Mitogenic for smooth muscle cells Inhibits endothelial cells, keratinocytes and lymphocytes Regulates matrix proteins, including collagen, proteoglycans, fibronectin and matrix degrading proteins. Stimulate proliferation of fibroblasts and production of collagen I and III.
Vascular endothelial growth factor (VEGF)	Chemotactic and mitogenic for endothelial cells. Mediates angiogenesis
Epithelial growth factor (EGF)	Mediates angiogenesis Mitogenic for fibroblasts, endothelial cells and keratinocytes Promoting cell proliferation and recruitment
Hepatocyte growth factor (HGF)	Mediates regeneration
Fibroblast growth factor (FGFII) (FGF-9)	Mediates tissue organisation and regeneration Aids generation of new follicles

Platelets release a plethora of growth factors that potentially promote or inhibit angiogenesis and influence the reactivity of tissue repair (Gawaz, 2008). One such growth factor is vascular endothelial growth factor (VEGF) which is a potent angiogenic factor (Wartiovaara, 1998). These protein ligands (a molecule or a molecular group that binds to another chemical entity to form a larger complex) are known to regulate cell migration, vascularisation, cell proliferation, and deposition of new extracellular matrix (Barrientos, 2008).

The Role of Growth Factors

A growth factor is a naturally occurring substance capable of stimulating cellular growth, proliferation, healing, and cellular differentiation. Usually it is a protein or a steroid hormone. Growth factors are important for regulating a variety of cellular processes.

Growth factors typically act as signalling molecules between cells. Examples are cytokines and hormones that bind to specific receptors on the surface of their target cells.

They often promote cell differentiation and maturation, which varies between growth factors. For example, epidermal growth factor (EGF) enhances osteogenic differentiation, while fibroblast growth factors and vascular endothelial growth factors stimulate blood vessel differentiation (angiogenesis).

Growth factor is sometimes used interchangeably among scientists with the term cytokine.

The growth factor was first discovered by Rita Levi-Montalcini, which won her a Nobel Prize in Physiology or Medicine.

The alpha granules in blood platelets contain growth factors PDGF, IGF-1, EGF, and TGF-β which begin healing of wounds by attracting and activating macrophages, fibroblasts, and endothelial cells.

What is Platelet Rich Plasma (PRP)?

Platelet-rich plasma (PRP), also known as autologous conditioned plasma, is a concentrate of platelet-rich plasma protein derived from whole blood, centrifuged to separate the cells. Therefore, it is a concentration of platelets in a small amount of plasma so why the term “platelet-rich-plasma”(Robert, 2001). Normal platelet counts in blood range between 150,000/µl and 350,000/µl and average about 200,000/µ (Robert, 2001). PRP is obtained by firstly taking a small blood sample of blood by venepuncture from the person being treated using PRP vacutainer tubes which contain a gel a separator and 3.2% sodium citrate. The sodium citrate inhibits platelet activation. The blood is then placed in a centrifuge and spun at high speed, greater than 1400RCF. The protocols here vary again depending on what brand of PRP kit you use, and which commercial organisation sold it to you. This process separates platelets from other blood components. The concentration of platelets is then injected into the area of the person’s body that needs to be treated.

What is Fibrin

The use of growth factors and fibrin for regenerative purposes represents a new approach to aesthetic medicine. The process of tissue regeneration includes a complex set of biological events controlled by the action and synergy of a cocktail of growth factors. The three agents involved in tissue regeneration:

the cellular component, a combination of multiple biological mediators that include growth factors and cytokines and a fibrin matrix or ‘scaffold’ that provides tissue construction support and acts as a net to trap platelets and growth factors.

Fibrinogen is produced by the liver and found in blood plasma and platelet alpha-granules, it's a glycoprotein complex that circulates in the blood of vertebrates. During tissue and vascular injury, it is converted enzymatically by thrombin to fibrin. Fibrin also mediates blood platelet and endothelial cell spreading, tissue fibroblast proliferation, capillary tube formation, and angiogenesis and thereby promotes revascularization and wound healing.

Thrombin is a naturally occurring enzyme that converts fibrinogen into fibrin. It is the only factor capable of cleaving fibrinogen to create fibrin.

Bacteriostatic potential is due to fibrin and not just leukocytes.

The three-dimensional cross-linked matrix serves as a template or scaffold for the organization of platelets, growth factors, leukocytes, cytokines, and mesenchymal stem cells during the remodelling of tissues. It massively traps the platelets and controls their release of growth factors “very slowly and continuously over a time period of more than one week”. Growth factors release rate into the target tissue is important for tissue receptors to respond over a prolonged period following treatment.

Fibrin also binds to Hyaluronic Acid which stimulates human fibroblast proliferation within a collagen matrix.

What is i-PRF?

i-PRF (Injectable Platelet Rich Fibrin) is a second-generation blood concentrate which is obtained at lower centrifuged speed and without anticoagulants (Dohan, 2006). Fibrinogen and von Willebrand factor (VWF) are the major ligands that form bridges that crosslink platelets together (Anituam 2004). The administration of a fibrin clot or fibrin glue contributes to an adhesive support that can confine secretion to a chosen site (Anitua, 2004). In i-PRF the growth factors are released from platelets. The centrifugation is at low speed which result in the preservation of platelets and leukocytes, allowing a greater harvest of cells. The leukocytes along with platelets play a key role in wound healing and tissue regeneration (Davis, 2014) but also boosts the process in a highly effective way (Choukroun, 2017). The leukocytes along with the platelets secrete different growth factors and proinflammatory cytokines as well as mediating endothelial adhesion, migration, proliferation and formation of granulation tissue (Adams, 2007). Therefore, when tissue injury occurs, the platelets are activated change from disc shape to dendrite, and secrete different growth factors such as platelet derived growth factor (PDGF) and transforming growth factor β (TGFβ) essential for initiation of inflammatory process by mediating recruitment and activation of immune cells (leukocytes) (Martin 1997). The activated platelets aggregate at site of injury and form a primary platelet plug which at this state is not yet stable until they adhere to fibrinogen forming a fibrin matrix.

The fibrin matrix is a complex 3-dimensional architecture necessary to increase tissue thickness. Interestingly advancements and the development of injectable platelet rich fibrin utilise the newly formed fibrin strands within the plasma to trap the platelets and leukocytes and slowly release the growth factors. Furthermore, platelets are activated during this process and leads to substantial embedding or platelet and leukocyte growth factors into the fibrin matrix (Dohan, 2006). Therefore, the differences between PRP and i-PRF is the activation of platelets, slow release of growth factors and higher leukocyte content. Dohan (2008) continues that leukocytes plays a role in proliferation, differentiation, immunity and infection. However, the main advantage of i-PRF is the fact that the fibrin matrix anchors the platelets.

Wang (2016) continues that one of the drawbacks of PRP is that the additional use of anticoagulants within PRP inhibit wound healing and platelet activation. They then tested their findings in a comparative study of the influence of PRP and i-PRF on osteoblast migration, proliferation and differentiation. What they did find is that PRP releases most of their growth factors at very early periods compared to i-PRF which produces a more gradual and sustained release of growth factors (Foster, 2009). I-PRF releases growth factors slower due to the use of a fibrin scaffold that entraps growth factors and releases them over time as well as housing leukocytes which responsible for additional growth factor release (Kobayasi, 2016). In two other articles they further argue that by decreasing the centrifugation speeds, a higher proportion of leukocytes can further increase the total growth factor release (Fujioka-Kobayashi, 2016, Ghanaati 2014).

Cell and Tissue Research

Platelets are cytoplasmic fragments of megakaryocyte, large cells formed in the bone marrow. Platelets are the smallest of the cellular elements in blood. The rate of platelet formations is governed by the amount of oxygen in the blood and the presence of nucleic acid derivatives from injured tissue (Free Dictionary, 2020). Platelets have been widely used in a variety of surgical procedures such as maxillofacial bone defects, orthopaedic and gastro surgery and now dermatology (Dae, 2011). In aesthetics the focus is on ageing human skin. Ageing is attributed to a combination of intrinsic and extrinsic factors. These factors influence and changes the physiological structure of the skin and its layers in the following ways. The skin cycle lengthens as we age with slow epidermal turnover (Ganceviciene 2012) in combination with acceleration of matrix metalloproteinase (MMP) (Bowes 2012). MMP is an enzyme that breaks down extracellular matrix (collagen, elastin and glycosaminoglycans (GAGs) (Bowes 2012). Three essential components makes up the bulk and support system of the extra cellular matrix (ECM) which are: collagen, elastin and glycosaminoglycans (GAGs) (Lee 2019). Collagen provides skin structure and integrity (Poon 2014) which consist of long fibres of protein and 29 types exist but the majority of collagen are Type I, III and V (Lee 2019). The main function of collagen is to provide strength to the skin and hold the skin together (Wohrle, 2019). GAGs link to water and fills the space between collagen and elastin fibres increasing skin turgidity (Lee 2019). Collagen is dependent on the individual skin type and any injury to the skin (Poon 2014). Aged

skin has fragmented elastic fibres with decreased and disproportionate collagen especially in type I and III (Gao 2008) The breakdown of GAG (water binding substance) result in skin dryness, laxity and increased wrinkling (Wohrle 2019). The slower skin cell turnover also affects healing and desquamation which results in dull and uneven skin appearance (Ganceviciene 2012). Histologically ageing skin has a flattened dermo-epidermal junction, dermal atrophy and fewer fibroblasts (Shin, 2012). One of the most important factors in preventing ageing skin is to target fibroblasts and collagen (Banihashemi, 2014). The interaction between fibroblasts and keratinocytes, adipocytes and mast cells are important in the skin ageing process as they are loaded with ECM, proteins, glycoproteins, adhesive molecules and cytokines (Shin, 2012). This interaction produces molecules to strengthen cell interaction and participated in fibroblast – keratinocyte – endothelium axis that preserves skin integrity and youth (Le Pillouer-Prost, 2003).

The various growth factors and cytokines that facilitate ECM accumulation and improve cell proliferation and differentiation are activated after injection into the target tissue (Banihashemi, 2014). Dermal fibroblasts are the source of extracellular matrix (ECM) proteins, glycoproteins, adhesive molecules and cytokines (Le Pillouer-Prost, 2003). Strategies such as mesotherapy, PRP/ i-PRF, micro needling, laser and topical treatments aim to increase ECM synthesis through activation of fibroblasts (Dae, 2011). Dermal fibroblasts play a key role in aging process through their interactions with keratinocytes, adipocytes and mast cells (Dae, 2011). Fibroblasts express numerous surface receptors and can simultaneously sense multiple molecules that trigger behavioural responses (Anitua, 2009). Growth factors such as (FGF1) and cytokines preserves the skin texture and are important in the regeneration and proliferation process of skin cells, including accumulation of collagen type 1 alpha 1 protein in skin and inhibition of UV-induced skin damage (Kakudo, 2008, Kim, 2011). Therefore, skin elasticity is also greatly improved. Cell rich plasma increases the expression of MMP-1 and MMP – 3 protein and may cause MMP remodelling through stimulating the removal of photo-damaged ECM components and inducing synthesis of new collagen by fibroblasts, which are in turn proliferated by their stimulation (Kim, 2011). Furthermore, fibrin binds to platelets and hyaluronic acid. Hyaluronic acid stimulates fibroblasts. accelerates hyaluronic acid production, which in turn means more water absorption and therefore increased skin volume and turgor (Kakudo, 2008). i-PRF is used for both stimulation of superficial and deep dermis layers. i-PRF can be utilised as a substitute to enhance tissue regeneration, speed wound healing and enhance collagen synthesis.

Neutrophils in tissue injury and repair - Neutrophils are the predominant immune cell in human blood, where they patrol and protect the host from pathogens and other harmful reagents (Kolaczkowska and Kubes 2013). In cases where the inflammatory process is generated by injury itself, which is also known as sterile inflammation, it becomes more controversial as to whether neutrophils themselves have any beneficial effects that may contribute to repair the parenchyma or vasculature. The outcome of the neutrophil response will most likely to be context-dependent, which includes but is not limited to, the trigger of the inflammatory response, the tissue environment and other cell types that interact with neutrophils. Altogether, these factors collectively determine whether an inflammatory response is a positive feedback amplification progress or a negative feedback self-limiting progress.

Importantly, it is still largely unknown how these progresses are determined and the key mediators that trigger the conversion from physiological tissue repair and regeneration to pathological tissue damage and chronic diseases remains to be defined. However, it has been only recently that researchers have started moving forward, looking at neutrophil dynamics and events after their recruitment to the tissue.

Mature neutrophils have more than 700 proteins including growth factors or pro-angiogenic factors stored in their segmented nucleus and granules (Dalli et al. 2013). Many can be rapidly released upon activation independent of transcription and thus directly contribute to regeneration and revascularization. The most widely studied mechanism of neutrophil contribution to tissue repair is that neutrophils become apoptotic and are cleared by macrophages (Soehnlein and Lindbom 2010). This clearance process initiates a feed-forward pro-resolution programme that is characterized by the release of the tissue-repairing cytokines transforming growth factor-β (TGFβ) and interleukin-10 (IL-10). Thus, drugs that promote neutrophil apoptosis have a therapeutic potential to accelerate tissue repair (Robertson et al. 2014).

The Use of Platelet Treatment in Hair Rejuvenation

A common chronic hair loss disorder is known as androgenic alopecia (AGA) affects 80% of men and 40% of women (Gentile 2015), it is the most common type of hair loss and occurs due to two prime reasons genetic predisposition and hormonal stimulation.

A meta-analysis of the evidence of the use of PRP in hair restoration appeared to be compelling (Giordano, 2017). Marwah et al. stated that PRP provides 300%–700% enrichment of platelets. However, its optimal concentration is still unclear. According to Giordano (2017) a significant increase of hairs per cm2 was observed after treatment with PRP. The VEGF8 and PDGF4 growth factors are known to facilitate angiogenesis around the hair follicle which can foster hair growth (Reese, 2010). The growth factors already discussed in paper acts on the stem cells in the bulge area of the follicles, stimulating the development of new follicles and promoting neovascularisation (Khatu, 2014). Hair growth with i-PRF has better regenerative potential. Its three-dimensional fibrin network mimics the extracellular matrix in terms of its structure, which creates the environment for cells to function optimally (Dohan 2012).

The Different Classifications of PRP / i-PRF

Nomenclature with Regards to Classification of Platelet Concentrations (Arshdeep, 2014)

Classification of Preparation	**Explanation**
P-PRP – Pure Platelet Rich Plasma	Undetermined buffy coat, containing platelets but most leukocytes and RBC are not collected.

PPP – Platelet Poor Plasma	Blood plasma with very low number of platelets (< 10 X 103/μL). Traditionally, PPP was recommended for use in platelet aggregation studies to both adjust the Platelet-rich plasma concentration, and to serve as a control.
L-PRP – leukocyte – and platelet rich plasma	Consists of most platelets along with leucocytes and residual red blood cells (RBC) suspended in fibrin-rich plasma. It differs from PRP on the means of buffy coater layer collection
P-PRF – pure platelet rich fibrin or synonym of platelet rich fibrin matrix (PRFM),	P-PRF is mixed with activator and allowed to incubate for some time, a stable PRFM clot can be collected and used.
L-PRF – Leukocyte and platelet rich fibrin.	Blood is collected and immediately centrifuged. A natural coagulation process then occurs and allows for easy collection of leucocytes and platelet rich fibrin (L-PRF) without any biochemical modification of the blood such as anticoagulants, thrombin or calcium chloride. L-PRF is used to stimulate bone formation to facilitate ideal placement of implants.
PRFM – Platelet Rich Fibrin Matrix	Supplier use the same principle of L-PRF but calls it something else to distinguish themselves from others in the field.
i-PRF – Injectable Platelet Rich Fibrin	Liquid formation without the use of additives. Contains a greater proportion of blood plasma and leukocytes due to low spin speed. Clinically applicable cell-based tissue engineering.
A-PRF – Advanced Platelet Rich Fibrin	Based on the low speed centrifugation concept for more rapid and thorough vascularization and wound healing.

All PRPs are not equal (Reese, 2010). There is yet no definitive protocol in the 3 modalities (PRP/PRF/ i-PRF) with regards to amount of treatments required to achieve best results (Shin, 2011, Yuksel, 2014, Nofal, 2014). There are many concepts in the field of PRP that needs clear definition to exclude the confusion in nomenclature. Differences in PRP results exist due to differences in the way samples are retrieved and vary from person to person (Giordano, 2017). Different PRP systems utilise different centrifugation chambers and spin parameters that dramatically impact on the product they produce (Roukis, 2006). This affects the force time and platelet density being different with a variation in growth factor concentrations (Han, 2007). Furthermore, the end products vary with regards to number or absence of platelets, leukocytes and protein content, colour and number of injections (Wroblewski, 2010). This is evident in our evaluation. As a result, variation in clinical results exist with various inconsistencies (Han, 2007). Han (2007) continues that the ideal with regards to platelet and growth factor concentrate is yet to be determined.

Conclusion:

The evaluation proved that the i-PRF preparation conditions and protocol yielded a significantly higher platelet yield compared to the PRP protocols. The literature review has demonstrated the effectiveness and function of platelets. What this evaluation further demonstrates is that commercial entities and clinicians are confused by the nomenclature and do not have a proper understanding or a grasp on what their various centrifuges deliver and its subsequent impact on their results. In this study lower centrifugation forces delivered higher platelet counts. This question the future use of PRP and its effectiveness. Is the second-generation blood concentrate i-PRF the better option for clients and clinicians?

Methods and products need definition and standardisation, specific quality parameters and clinical indications, including contraindications (Banihashemi, 2014). Limited studies exist on clinical efficacy and safety. This study goes some way to highlight the differences between PRP and i-PRF. However, more studies are needed to test effectiveness in practice.

Clinical Relevance:

The variation of platelet and other blood component concentrations between commercial PRP kits may affect clinical treatment outcomes. There is a need for standardization of PRP for clinical use. i-PRF goes some way to offer standardisation and consistency with a simple and effective protocol which offers the highest platelet concentration.

References:

Abrams, C.S., Brass, L.F. (2001) *Platelet signal transduction*. In: Homeostasis and Thrombosis. Basic Principles and Clinical Practice. Colman, R.W., et al., eds. Lippincott, Williams & Wilkins: Philadelphia. 541 - 559.

Adams, R.H., Alitalo, K. (2007) Molecular regulation of angiogenesis and lymphangiogenesis. *Nature Reviews Molecular Cell Biology*. Volume 8, Issue 6: p 464 – 478.

Anitua, E., Andia, I. et al, (2004) Autologous platelets as a source of proteins for healing and tissue regeneration. *Thromb Haemostat.* Volume 91: p 4 – 15.

Anitua, E., Sanchez, M., et al, (2009) Fibroblastic response to treatment with different preparations rich in growth factors. *Cell Proliferation.* Volume 42: 162 – 170.

Arshdeep, M., Kumaran, S. (2014) Platelet-rich plasma in dermatology: Boon or bane? *Indian Journal of Dermatology, Venereology and Leprology*. Volume 80, Issue 1: p. 5 – 14.

Banihashemi, M., Nakhaeizadeh, S. (2014). An introduction of platelet rich plasma (PRP) in skin rejuvenation. *Review Clin Med.* Volume 1: 38 – 43.

Barrientos, S., et al, (2008) Tomic-canic growth factors and cytokines in wound healing. *Wound Rep Regen*. Volume 16: 585 – 601.

Boswell, S.G., Schnabel, L., et al, (2014) Increasing platelet concentrations in leukocyte-reduced platelet -rich plasma decrease collagen synthesis in tendons. *The American Journal of Sports Medicine.* Volume 42: No.1: p. 42 – 49.

Bowes, L (2012) Exploring the science of cosmeceuticals used to achieve optimal skin health. *Journal of Aesthetic Nursing.* Volume 1, Issue 5: 232 – 241.

Bukhari, S.A., (2011) What is a comparative study? SSRN "growth factor" at Dorland's Medical Dictionary

file:///C:/Users/tania/AppData/Local/Packages/Microsoft.MicrosoftEdge_8wekyb3d8bbwe/TempState/Downloads/SSRN-id1962328%20(1).pdf Accessed: 24/01/2020.

Choukroun, J., Ghanaati, S (2017) Introducing the low-speed centrifugation concept. In: *Platelet Rich Fibrin in Regenerative dentistry: Biological Background and Clinical Indications.* 1st Ed. Miron, R.J., Choukroun, J. John Wiley & Son's Limited,

Clausen, C., et al, (2006) Homologous activated platelets stimulate differentiation and proliferation of primary human bone cells. *Tissues, Bones, Organs.* Volume 184: 68 – 75.

Dae, H.K., Young, J.J. et al, (2011) Can platelet rich plasma be used for skin rejuvenation? Evaluation of effects of platelet rich plasma on human dermal fibroblast? *Ann Dermatol*. Volum 23, Issue 4: p. 424 – 431.

Dalli J, Montero-Melendez T, Norling LV, Yin X, Hinds C, Haskard D, Mayr M, Perretti M. Heterogeneity in neutrophil microparticles reveals distinct proteome and functional properties. Mol Cell Proteomics. 2013;12:2205–2219. doi: 10.1074/mcp.M113.028589. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

Davis, V., Abukabda, A.B., Radio, N.M., et al, (2014) Platelet rich preparations to improve healing. Part II: Platelet activation and enrichment, leukocyte inclusion, and other selection criteria. *Journal of Oral Implantology*. Volume 40 Issue 4: 511 – 521.

Del Angel-Mosqueda C, Gutiérrez-Puente Y, López-Lozano AP, Romero-Zavaleta RE, Mendiola-Jiménez A, Medina-De la Garza CE, Márquez-M M, De la Garza-Ramos MA (September 2015). "Epidermal growth factor enhances osteogenic differentiation of dental pulp stem cells in vitro". Head & Face Medicine. 11: 29. doi:10.1186/s13005-015-0086-5. PMC 4558932. PMID 26334535

Dohan, D.M., et al (2006) *Platelet Rich Fibrin (PRF): a second-generation platelet concentrate. Part 1: technological concepts and evolution.* Oral Surgery, Oral Med, Oral Pathology. Oral Radiolody. Volume 103: p. 587 – 593.

Dohan, D.M., Rasmusson, L. et al (2009) *Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte – and platelet-rich fibrin (L-PRF).* Trends in Biotechnology. Volume 27, Issue, 3: p. 158 – 167.

Dohan Ehrenfest DM, Bielecki T, Jimbo R, Barbé G, Del Corso M, Inchingolo F, Sammartino GCurr Pharm Biotechnol. 2012 Jun; 13(7):1145-52.

Dohle, E., El Baghdadi, K., et al, (2018) Platelet-rich fibrin-based matrices to improve angiogenesis in an in vitro co-culture model for bone tissue engineering. *Journal Tissue Eng Regen Med.* Volume 12: 598 – 610.

Marwah M, Godse K, Patil S, Nadkarni N. Is there enough research data to use platelet-rich plasma in dermatology? Int J Trichology. 2014;6:35–6. [PMC free article] [PubMed] [Google Scholar]

Foster, T.E., Puskas, B.L. (2009) Platelet rich plasma: from basic science to clinical application. *The American Journal of Sports Medicine.* Volume 37, Issue 11: p. 2259 – 2272.

Free Dictionary (2020) Platelet. https://medical-dictionary.thefreedictionary.com/platelet+release+reaction Accessed: 29/01/2020.

Fujioka – Kobayashi, M. Miron, R.J. et al, (2016) Optimised platelet rich fibrin with the low speed concept: grown factor release, biocompatibility and cellular response. *Journal Periodontol.* P. 1- 17.

Ganceviciene, R.L.A., Theodoridis, A, et al, (2012) Skin anti-ageing strategies. *Dermato-Endocrinology*. Volume 4, Issue 3: p308 – 319.

Gawaz, M., Stellos, K., Langer, H.F., (2008) Platelets modulate atherogenesis and progression of atherosclerotic plaques via interaction with progenitor and dendritic cells. *Journal Thromb Haemost.* Volume 6, Issue 2: 352 – 242.

Gentile, P., Garcovich, S., et al, (2015) The effect of platelet rich plasma in hair regrowth: a randomised placebo-controlled trial. *Stem Cells Translational Medicine*. Volume 4, Issue 11: p. 1317 – 1323.

Gibble, J.W., Ness, P.M., (1990) Fibrin glue: the perfect operative sealant? *Transfusion*. Volume 30, p. 741 – 747.

Giordano, S., Romeo, M., et al, (Platelet-rich plasma for androgenetic alopecia: Does it work? Evidence from meta-analysis. *J Cosmetic Dermatol*. Volume 16: 374 – 381.

Ghanaati, S., Booms, P., et al, (2014) Advanced platelet rich fibrin: a new concept for cell-based tissue engineering by means of inflammatory cells. *Journal Oral Implantology*. Volume 40, Issue 6: 679 – 689.

Han, J., et al, (2007) The effect of different platelet-rich plasma concentrations on proliferation and differentiation of human periodontal ligament cells in vitro. *Cell Proliff.* Volume 40: 241 – 252.

Kakudo, N., Minakata, T., et al, (2008) Proliferation – promoting effect of platelet rich plasma on human adipose-derived stem cells and human dermal fibroblasts. *Plastic Reconstructive Surgery.* Volume 122: p. 1352 – 1360.

Kawazoe, T., Kim, H.H., (2012) Tissue augmentation by white blood cell-containing platelet-rich plasma. *Cell Transplant.* Volume21: p.601 – 607.

Khatu, S.S., More, Y.E., et al, (2014) Platelet rich plasma in androgenic alopecia: myth or an effective tool. *Journal Cutaneous Aesthetic Surgery.* Volume 7: p. 107 – 110.

Kim, D.H., Kim C.D., et al, (2011) Can platelet rich plasma be used for skin rejuvenation? Evaluation of effects of platelet rich plasma on human fibroblast. *Annual Dermatology.* Volume 23: p. 424 – 431.

Kobayasi, E., Fluckiger, L., et al, (2016) Comparative release of growth factors from PRP, PRF and advanced PRF. *Clinical Oral Investigation.* Volume 20, Issue 9: p. 2353 – 2360.

Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. Nat Rev Immunol. 2013;13:159–175. doi: 10.1038/nri3399. [PubMed] [CrossRef] [Google Scholar]

Lancaster, T.D., Montinola, G.R. (1997) Toward a methodology for the comparative study of political corruption. *Crime, Law & Social Change*. Volume 27: 185 – 206.

Lee, R. (2019) Collagen, Elastin and Glycosaminoglycans. *Just About Skin.*

Le Pillouer-Prost, A. (2003) Fibroblasts: what's new in cellular biology? *Journal of Cosmetic Laser Therapy*. Volume 5: p. 232 – 238.

Li, Z.J., Choi, H.I, et al, (2012) Autologous platelet-rich plasma: A potential therapeutic tool for promoting hair growth. *Dermatol Surg.* Volume 38: p 1040 – 1046.

Lin, F., Butcher, E.C., (2008) Modeling the role of homologous receptor desensitisation in cell gradient sensing. *Journal Immunology.* Volume 181: 8335 – 8343.

Martin, P. (1997) Wound healing-aiming for perfect skin regeneration. *Science*. Volume 276, Issue 5309: p. 75 – 81.

Matras, H. (1970) Effect of various fibrin preparations on re-implantations in the rat skin. *Osterr Z. Stomatol.* Volume 67, Issue 9: p. 338 – 359.

McCarrel, T., Minhas, T., Fortier, L. (2012) Optimisation of leukocyte concentrations in platelet rich plasms for the treatment of tendinopathy. *Journal Bone Joint Surg Am* Volume 94, Issue 19: e, 143: p 1 – 8.

Miron, R., Fujioka-Kobayashi, M., et al, (2016) Platelet rich fibrin and soft tissue wound healing: a systematic review. *Tissue Eng Part B Review.* Volume 23, Issue 1: 83 – 99.

Nofal, E., Helmy, A, et al, (2014) Platelet rich plasma versus CROSS technique with 100% trichloraecetic acid versus combined skin needling and platelet rich plasma in the treatment of atrophic acne scars: a comparative study. *Dermatological Surgery.* Volume 40: p. 864 – 873.

Pickvance, C. (2005). The four varieties of comparative analysis: the case of environmental regulation. Conference paper on small and large – N comparative solutions. http://eprints.ncrm.ac.uk/57/1/chrispickvance.pdf (Access: 24/01/2020).

Poon, F.K.S., Chien, A.L., (2014) Mechanisms of Treatments of Photoageing. *Photodermatology, Photoimmunology & Photomedicine.* Volume 31: p. 65 – 74.

Quan, T., Qin, Z., et al, (2009) Matrix-degrading metalloproteinases in photoageing. *Journal of Investigatory Dermatology.* Sympo Proc. Volume 14: 20 – 24.

Redaelli, A., Romano, D., Marciano, A. (2010) *Face and neck revitalization with platelet rich plasma (PRP): clinical outcome in a series of 23 consecutively treated patients.* Journal Drugs Dermatology. Volume 9: 466 – 472.

Reese, R.J., (2010) Autologous platelet rich plasma (PRP): what do we know? Important concepts relevant to hair restoration surgery. Hair Transplant Forum International. https://pdfs.semanticscholar.org/c55b/61f39ff986b326d37468fcba6705e2551f4e.pdf Accessed: 29/01/2020.

Robert, M. (2001) *Platelet Rich Plasma (PRP):* What is PRP and What is Not PRP*? Implant Dentistry*. Volume 10, Issue 4: p 225 – 228.

Robertson AL, Holmes GR, Bojarczuk AN, Burgon J, Loynes CA, Chimen M, Sawtell AK, Hamza B, Willson J, Walmsley SR, et al. A zebrafish compound screen reveals modulation of neutrophil reverse migration as an anti-inflammatory mechanism. Sci Transl Med. 2014;6:225ra229. doi: 10.1126/scitranslmed.3007672. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

Roukis, T.S., Zgonis, T., et al, (2006). Autologous platelet rich plasma for wound and osseous healing: a review of the literature and commercially available products. *Advances in Therapy. Volume 23, Issue 2: p. 218 – 237.*

Rughetti, A., et al, (2008) Platelet gel-released supernatant modulates and antiogenic capability of human endothelial cells. *Blood Transfusion*. Volume 6: 12 - 17

Shin, M.K., Lee, J.H., et al, (2012) Platelet rich plasma combined with fractional laser therapy for skin rejuvenation. *Dermatol Surgery.* Volume 38: p. 623 - 630

Smelser, N.J., (1976) *Comparative Methods on the Social Sciences.* Prentice-Hall, Englewood Cliffs.

Soehnlein O, Lindbom L. Phagocyte partnership during the onset and resolution of inflammation. Nat Rev Immunol. 2010;10:427–439. doi: 10.1038/nri2779. [PubMed] [CrossRef] [Google Scholar]

Steed, D.L., (1997) The role of growth factors in wound healing*. Surgical Clinical North America*. Volume 77: p. 575 – 86.

Stellos, K., Kopf, S., Paul, A., et al, (2010) Platelet Regeneration. *Seminars in Thrombosis and Haemostasis*. Volume. 36, No. 2: p. 175 – 183.

Takakura, N., Yoshida, H. et al, (1996) Involvement of platelet-derived growth factor receptor-alpha in hair canal formation*. Journal Investigative Dermatology*. Volume 107: p.770 – 7.

Talwar, H.S., Griffiths, C.E., (1995) Reduced type I and type III procollagens in photo damaged human skin. *Journal Investigative Dermatology.* Volume 105: p. 285 – 290.

Tilly, C. (1984) *Big Structures, Large Processes, Huge Comparisons.* Russel Sage Foundation, New York.

Trink, A., Sorbellini, E., et al, (2013) A randomised, double blind, placebo and active-controlled, half head study to evaluate the effects of platelet rich plasma and alopecia areata*. Br J Dermatol.* Volume 169: p. 690 – 694.

Wang, X., Zhang, Y., Choukroun, J. et al (2017) Effects of an injectable platelet-rich fibrin on osteoblast behaviour and bone tissue formation in comparison to platelet-rich plasma*. Platelets*. Volume 29 p. 48 – 55.

Wartiovaara, U., Salven, P, et al, (1998) Peripheral platelets express VEGF-C and VEGF which are released during platelet activation. *Thromb Haemost.* Volume 80, Issue 1: p.171 – 175.

Worhle, M. (2019) Glycosaminoglycans (GAGs). *Truth in ageing: The honest truth about beauty & personal care products.*

Wroblewski, A.P., Melia, H.J. et al, (2010) Application of platelet rich plasma to enhance tissue repair. *Oper Tech Orthop*. Volume 20: p. 98 – 105.

Wu, D.T., et al, (2005) Concentration specifies differential signalling of growth arrest/differentiations and apoptosis in podocytes. *I Am Soc Nephrol.* Volume 16: 1 – 11.

Yildiz, O.K.A.H., Baloglu, H et al, (2016) Histologic evidence of new collagen formulation using platelet rich plasma in skin rejuvenation: A prospective controlled study. *Annual Dermatology.* Volume 28, Issue 6: p. 718 – 724.

Yuksel, E.P., Sahin, G, et al, (2014) Evaluation of effects of platelet rich plasma on human facial scar. *Journal Cosmetic Scar.* Volume 16: 206 – 208.

Yorio T, Clark AF, Wax MB (15 October 2007). Ocular Therapeutics: Eye on New Discoveries. Academic Press. pp. 88–. ISBN 978-0-12-370585-3. Retrieved 28 November 2010.