Platelet-Rich Plasma & Autologous Blood Concentrating Devices

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Abstract

The purpose of this review is to evaluate and understand the various preparation and separation parameters for Platelet-rich Plasma (PRP) use in dermatological settings. Further, this evidence-based assessment is intended to aid in the standardization and classification of PRP research parameters, provide background information related to molecular and biological mechanisms of PRP, and offer insight regarding clinical standards and best practices when choosing a PRP concentrating system for your practice and patients.

Overview

Platelet-rich plasma (PRP) is platelet concentrate prepared through centrifugation of autologous whole blood and is used in many different clinical point-of-care and surgical applications (Table 1). There are numerous PRP preparation systems available on the market and each generates a unique composition of platelets, growth factors, erythrocytes (red blood cells, or RBCs), and leukocytes (white blood cells, or WBCs). The efficacy of PRP highly depends on the values of individual blood components present in the final PRP concentrate. Variability in PRP formulations and concentrating devices derives from each commercial product's characteristic centrifugation parameters, manufacturing materials, initial blood volume, choice of anticoagulant and platelet activator, temperature and pH conditions, and extraction and cycle technique used to generate the PRP. Although there are many well-documented benefits of PRP in the literature, a standardized system for researching PRP formulations and their effect on condition specific treatment indications has not been developed. First, we examine the mechanism of action of PRP in tissue regeneration and repair in dermatologic and aesthetic applications. Next, we provide a comprehensive summary of the different preparation parameters that contribute to the variability of PRP formulations, followed by a summary of these applications to the IncMD c-PRP system. Throughout this assessment we provide PRP insight and best practices to maintain and exceed the standard of care afforded to your patients.

Mechanism of Action

Platelet α-granules, dense granules, lysosomes and surface adhesion molecules all contribute to the underlying cellular signaling mechanisms that render platelet regenerative function (Figure 1). Most of the PRP literature, however, has focused on the role of platelet α-granules, which contain cytokines, chemokines, growth factors and other low molecular weight proteins involved in cell proliferation and differentiation (Table 2) (Kang et al., 2014; Lee et al., 2012; Eppley et al., 2006; Everts et al., 2020). Once activated, platelets degranulate and release their contents, which results in the initiation of cellular signaling cascades that promote tissue regeneration and repair. Platelet growth

factors bind to surface receptors on osteoblasts, endothelial cells, stem cells, fibroblasts and other tissue-specific cells to activate intracellular signaling cascades resulting in the expression of genes and proteins that effectuate angiogenesis, collagen synthesis, decreased apoptosis and synthesis of extracellular matrix components (Mazzocca et al., 2012; Browning et al., 2012; Freire et al., 2012; Cho et al., 2011; Alsousou et al., 2009). The proposed mechanisms of PRP in cosmetic, dermatologic and aesthetic applications vary depending on the tissue site and location of use. (Pierce et al., 1989; Alsousou et al., 2009; Garcia et al., 2005).

Erythrocytes

When preparing PRP, the number of RBCs should be negligible. Erythrocytes contain reactive oxygen species (ROS) and free radicals that produce harmful inflammation, initiate edema and cause pain during and after injection (Magalon et al, 2014). Moreover, RBCs deposit hemosiderin, which together with the release of free radicals, contaminate PRP and lower solution pH, thus, reducing platelet viability (Lei et al, 2009). For hair restoration, these combined effects create a catabolic environment that hinders the role of growth factors and induces telogen effluvium.

RBC contamination may occur during collection, centrifugation or extraction of PRP. If the chemical properties of the tube and spin conditions are unsuitable, RBCs may lyse and release their harmful byproducts, and oxidative stress may cause other RBCs to undergo eryptosis and inflammation (Everts et al, 2019). The number of erythrocytes in the final PRP product varies depending on many factors including, the presence of a serum gel separator, centrifugation parameters, manufacturing materials, initial blood volume, and choice of anticoagulant (Kumar et al, 2005). A major drawback of using tubes without a gel separator is that remnants of RBCs spill over and contaminate the PRP concentrate. Thus, many dermatologists and aesthetic facilities may choose a plasma-based extraction method containing a gel separator to meet their patient needs.

White Blood Cells

The most contentious topic among PRP investigators is in regard to the role of WBC components found in the final PRP concentrate. Many studies contend that leukocyte-rich PRP (LR-PRP), which contains an abundance of neutrophils and other granulocytes creates unfavorable catabolic conditions in tissue and prolongs the inflammatory process The quality of a PRP concentrating device is further revealed by its ability to achieve an appropriate ratio of platelets to WBCs, as well as individual concentrations of specific WBC components. Macrophages and monocytes play a fundamental role in repairing damaged cells and maintaining tissue integrity (Fernandez 2005). Thus, the presence of monocytes in PRP is beneficial to the cellular rejuvenation process. Other leukocytes---namely neutrophils, basophils and eosinophils—release proinflammatory metalloproteinases and other

cytokines that prolong inflammation, neutralize growth factors, damage non-injured tissue, degrade extracellular matrices, cause fibrosis and scarring (Kim 2015, Fitzpatrick 2017, Magalon 2014, DeLong 2012). Thus, the presence of granulocytes antagonizes the healing properties of PRP and should be minimized. Although the ratio of platelets to WBC's is important, the process and methods used to separate PRP provides equally significant insight about the quality of PRP systems in practice.

Corporate Compliance

The US Food and Drug Administration's (FDA) Center for Biologics Evaluation and Research (CBER) regulates the market for biologics products, like PRP, which fall under the guidelines in 21 CFR 1271 of the CBER Code of Regulations. Under these provisions, products like PRP are exempt and do not follow the FDA's conventional regulations that would otherwise require clinical research or animal studies conducted prior to introducing a new PRP system to the market (Beitzel K et al, 2014). However, because the autologous nature of PRP renders it a low-risk procedure, PRP concentrating devices are class II medical devices that require FDA 510(k) clearance. To maintain an FDA "cleared" as opposed to an FDA "approved" medical device status, the FDA requires that the PRP collection device be similar to an already approved device, or predicate, and clearance is limited to indications of the predicate. Since the only approved on-label use for PRP is mixture with autograft or allograft bone to enhance graft properties, any use of PRP outside this context is considered "off-label." Nonetheless, clinicians are free to use their best clinical judgement when applying PRP for an off-label procedure as long as the use is based on scientific knowledge that's grounded in sound medical evidence (Harm et al, 2015).

Manufacturing Materials

Tubes manufactured using crystal glass and polyethylene terephthalate (PET) allow for a more effective and uniform PRP collection, centrifugation and extraction process that maximize platelet and growth factor concentrations (Bowen et al, 2014). The inner crystal layer is more hydrophilic than plastic, contains more tightly packed molecules to minimize wall rigidity, keeps out moisture, and allows for the thixotropic separating gel to readily move throughout the tube. The outer PET layer provides firm support, minimizes evaporation of anticoagulant solution, extends shelf life and maintains a prolonged vacuum that aids in the blood collection process. Smaller diameter tubes also help minimize surface area to atmosphere ratio, which prevents diffusion of CO_2 out of plasma and thereby help maintain the system's physiological pH (Collins et al, 2021).

Sterilization and Depyrogenation

Different sterilization techniques are used to render PRP and other medical devices "sterile" which must not be confused with rendering them "pyrogen-free." A product that is *sterile* is void of any

viable microorganisms (Lechman et al. 1976, Ashok 1994), whereas a product that is pyrogen-free has undergone more rigorous cleansing and must be confirmed not to contain pyrogens using either a Rabbit Test or Limulus Amoebocyte Lysate (LAL) Test. Although conventional methods to render a product sterile may be effective at removing the bioburden, sterilization techniques are ineffective at eliminating pyrogens and endotoxins. Further, sterilization techniques may generate pyrogens in the process of eliminating bacteria, whereby bacteria may release endotoxins and other remnants as byproducts after they denature (Van Belleghem et al, 2016).

ANTICOAGULANTS

As a major parameter affecting platelet yield, morphology and function, the type of anticoagulant used has been overlooked by researchers in more than 53% of all PRP-related studies (Frautschi et al, 2017), which represents a major shortcoming in the PRP literature. Studies that have highlighted the impact of choosing the appropriate anticoagulant have demonstrated that Acid Citrate Dextrose Solution A (ACD-A) is the PRP gold standard (Araki 2012, Fukaya 2014, Wahlstrom 2007). Other researchers demonstrated ACD-A's effectiveness over alternative anticoagulants in maintaining platelet viability and morphology (Lei et al., 2009; Giraldo et al., 2015; Singh et al., 2018). Solutions treated with ACD-A were most successful in maintaining platelet and alpha granule structure, whereas other anticoagulants resulted in a greater number of lysed cells and PRP contaminants. Samples stained and viewed under a TEM microscope revealed functional and morphological features that ACD-A exhibited over other anticoagulants, as well as a lack of acellular debris and aggregates, which together thwart premature platelet activation (Singh, 2018).

These studies and others established ACD-A's capacity to function as a physiological buffer, maintaining a pH near 7.2 and providing optimal conditions to maximize platelet and growth factor concentrations (Arora, 2017). The concentration and effectiveness of growth factors are impacted by tissue pH, so having PRP concentrate buffered close to a physiologic range provides ideal conditions. Moreover, dextrose and other ingredients support platelet metabolism and viability, whereas citrate binds calcium and prevents premature coagulation (Arora, 2017). Together, ACD-A maintains ideal chemical properties to keep PRP buffered and intact, and thus, is the anticoagulant of choice for Inc-MD's c-PRP concentrating devices.

CENTRIFUGATION

The process of centrifugal separation of blood components is a major contributor to the quality of a PRP system's final product. Currently, a standardized system for evaluating PRP effectiveness does not clinically exist. There's significant variation of cellular and molecular components when measured in different PRP collecting devices but using the same centrifugation protocols. Moreover,

differences in centrifugal force, acceleration and spin time produce major disparities in overall yields, purity, concentration, viability, morphology and activation status of platelets (Fadadu et al., 2019). Spin protocol, shock absorbance and dampening ability further contribute to the final components found in PRP concentrate and the most important parameters are investigated below:

Single vs. Double Methodology

Studies conducted comparing different centrifugation methodologies and their effect on platelet, leukocyte, and growth factor concentrations concluded that single-spin centrifugation protocols are more optimal for achieving greater platelet and growth factor concentrations (Carofino et al., 2012; Mazzocca et al., 2012; Pachito et al., 2020).

Centrifugal Force, Acceleration and Speed

For products that utilize a gel separator, a greater force is required to overcome the gel barrier and all RBCs and some granulocytes to traverse the gel. It is essential to determine the optimal force at which the concentration of PRP is the highest without compromising its morphology. Studies have revealed that a high-speed centrifugation during a short duration is best for achieving high platelet and growth factor concentrations. This force may range from 1500 to 2000g when using a single centrifugation approach (Dhurat & Sukesh, 2014; Croisé et al., 2020).

Spin Duration

Since it is highly efficacious to rely on a high centrifugal force during preparation of the autologous blood PRP, caution must be taken when setting the duration of spin to avoid damaging the platelets' morphology. Studies have revealed that a duration ranging from 5 to 12 minutes of spin combined with the parameters chosen above is effective for maintaining platelet integrity (Croisé et al., 2020).

Inc-MD c-PRP Concentrating Systems

Inc-MD c-PRP Concentrating System devices are Class II medical devices with 510(k) clearance (510(k) Number: BK170136) and our device manufacturer maintains a quality management system that’s ISO 13485 certified. All c-PRP collection tubes are manufactured using Crystel-PET and contain a polymeric, thixotropic gel separator with an optimized specific gravity of 1.05g/cm^3 that helps maximize platelet, monocyte and growth factor concentrations while eliminating RBCs and WBCs. Inc-MD c-PRP devices undergo triple sterilization using Cobalt^{60} gamma irradiation technology, and subsequently undergo depyrogenation in a unidirectional dry heat tunnel. The quality and safety of our devices exceed the industry and regulatory standards.

Inc-MD's c-PRP Concentrating System provides a PRP collection apparatus that provides renders RBCs negligible. Our system is optimized to separate the RBCs from the final platelet mixture without causing RBCs to lyse during the preparation process. Everything from the materials used to manufacture our collection tubes to the centrifugal force, speed and rotor angle contribute to a seamless PRP preparation system that's unwavering in its elimination of RBCs. Other parameters and specifics about how the c-PRP system eliminates RBCs and other harmful components are further described throughout this document.

TABLES AND FIGURES

TABLE 1.

Dermatological PRP Applications	PRP Applications in Other Specialties
▪ Androgenic alopecia ▪ Acne scarring ▪ Skin rejuvenation ▪ Striae distensae ▪ Skin aging ▪ Wrinkles ▪ Melasma & dyspigmentation ▪ Hair transplantation ▪ Periocular circles	▪ Tendinopathy ▪ Muscle injury ▪ Bone remodeling ▪ Osteoarthritis ▪ Bone grafts ▪ Fat transfers ▪ Breast augmentation ▪ Wound healing ▪ Dental bone rejuvenation

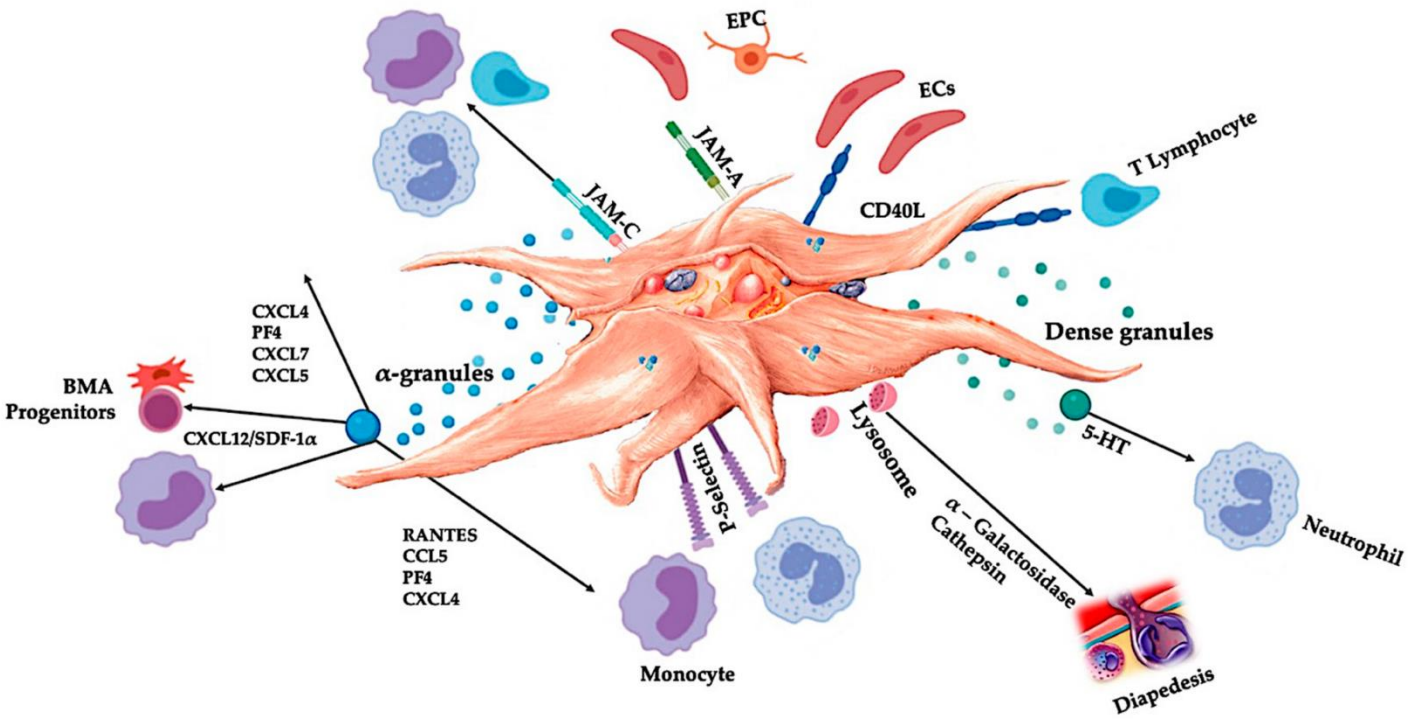
- PRP uses in dermatology and other medical specialties.

TABLE 2.

Growth Factor	Function
PDGFαα PDGF αβ PDGF ββ	▪ Chemotactic for fibroblasts, macrophages, & neutrophils ▪ Mitogenic for fibroblasts, endothelial cells, mesenchymal cells, smooth muscle cells & osteoblasts ▪ Promote collagen synthesis, and regulate collagenase secretion
TGF-β1	▪ Stimulate angiogenesis and proliferation of mesenchymal cells ▪ Regulate cell proliferation, differentiation, and apoptosis ▪ Chemotactic for fibroblasts, keratinocytes & macrophages ▪ Mitogenic for smooth muscle cells & fibroblasts ▪ Inhibit proliferation of keratinocytes, endothelial cells, lymphocytes & macrophages ▪ Regulate production of cellular matrix proteins
VEGF	▪ Increases vessel permeability & stimulates angiogenesis ▪ Chemotactic & mitogenic toward endothelial cells
FGF-2/7/9	▪ Help regenerate tissue ▪ Stimulate growth and differentiation of mesenchymal cells, osteoblasts & chondrocytes
EGF	▪ Regulates cell proliferation, differentiation & survival ▪ Stimulates angiogenesis, mitosis in fibroblasts, endothelial cells, mesenchymal cells & keratinocytes ▪ Promotes chemotaxis in endothelial cells ▪ Regulates secretion of collagenase
IGF-1	▪ Regulates cellular metabolism ▪ Stimulates protein synthesis; osteoblast proliferation & differentiation ▪ Chemotactic for fibroblasts
CTGF	▪ Promotes angiogenesis & regeneration of chondrocytes ▪ Involved in fibrosis & platelet adhesion
PDGF	▪ Fibroblast, macrophage, neutrophil chemotaxis ▪ Fibroblast, epithelial, smooth muscle, mesenchymal cell proliferation ▪ Collagen metabolism ▪ Angiogenesis

- **Growth factors contained within platelet granules and their various functions.**

FIGURE 1.

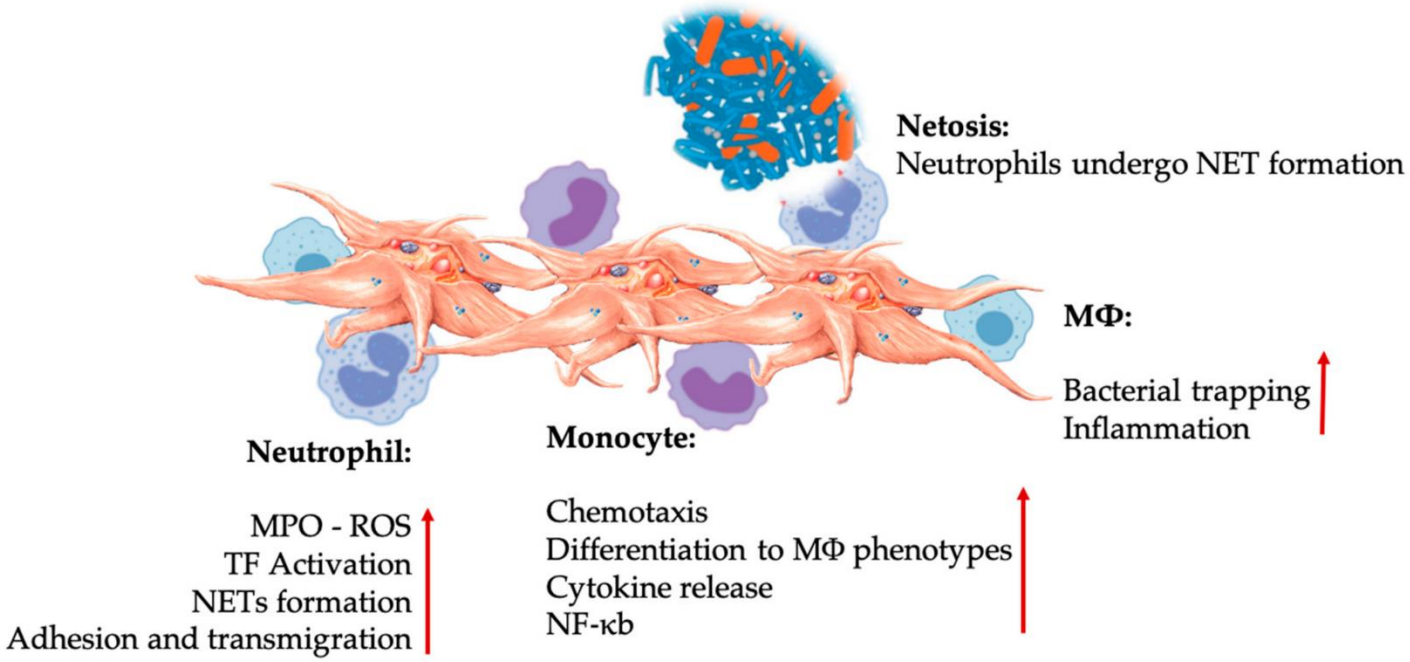


Adapted and Modified from Everts et al. and Herr et al.

- **Activated platelets, releasing PGF, and adhesion molecules mediate a variety of cellular interactions, like chemotaxis, cell adhesion, migration, cell differentiation, and immunomodulatory activities. These platelet cell-cell interactions contribute to angiogenesis and inflammatory activities, to ultimately stimulate tissue repair processes.**

Abbreviations: BMA: bone marrow aspirate, EPC: endothelial progenitor cell, EC: endothelial cells, 5-HT: serotonin, RANTES: Regulated upon Activation Normal T Cell Expressed and Presumably Secreted, JAM: junctional adhesion molecules type, CD40L: cluster of differentiation 40 ligand, SDF-1: stromal cell-derived factor 1 alpha, CXCL: chemokine (C-X-C motif) ligand, PF4: platelet factor 4.

FIGURE 2.

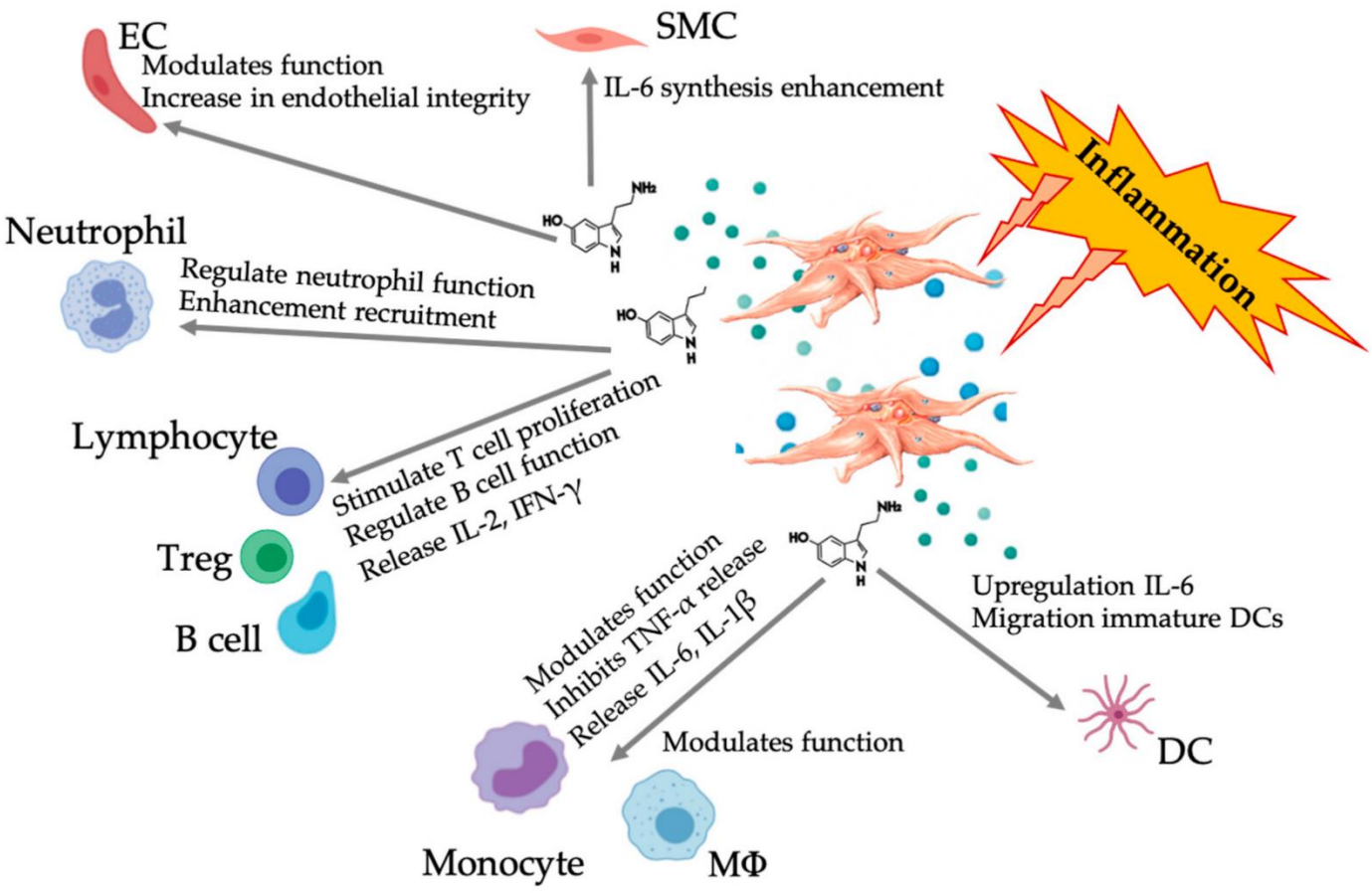


Adapted and Modified from Everts et al. and Herr et al.

- **Platelets communicate with neutrophils, monocytes, and Macrophages to modulate and increase their effector function. These platelet-leukocyte interactions result in inflammatory contributions through a variety of different mechanisms, including neutrophil extracellular traps (NETs).**

Abbreviations: MPO: myeloperoxidase, ROS: reactive oxygen species, TF: tissue factor, NET: neutrophil extracellular traps, NF-κB: nuclear factor kappa B, MΦ: macrophage.

FIGURE 3.

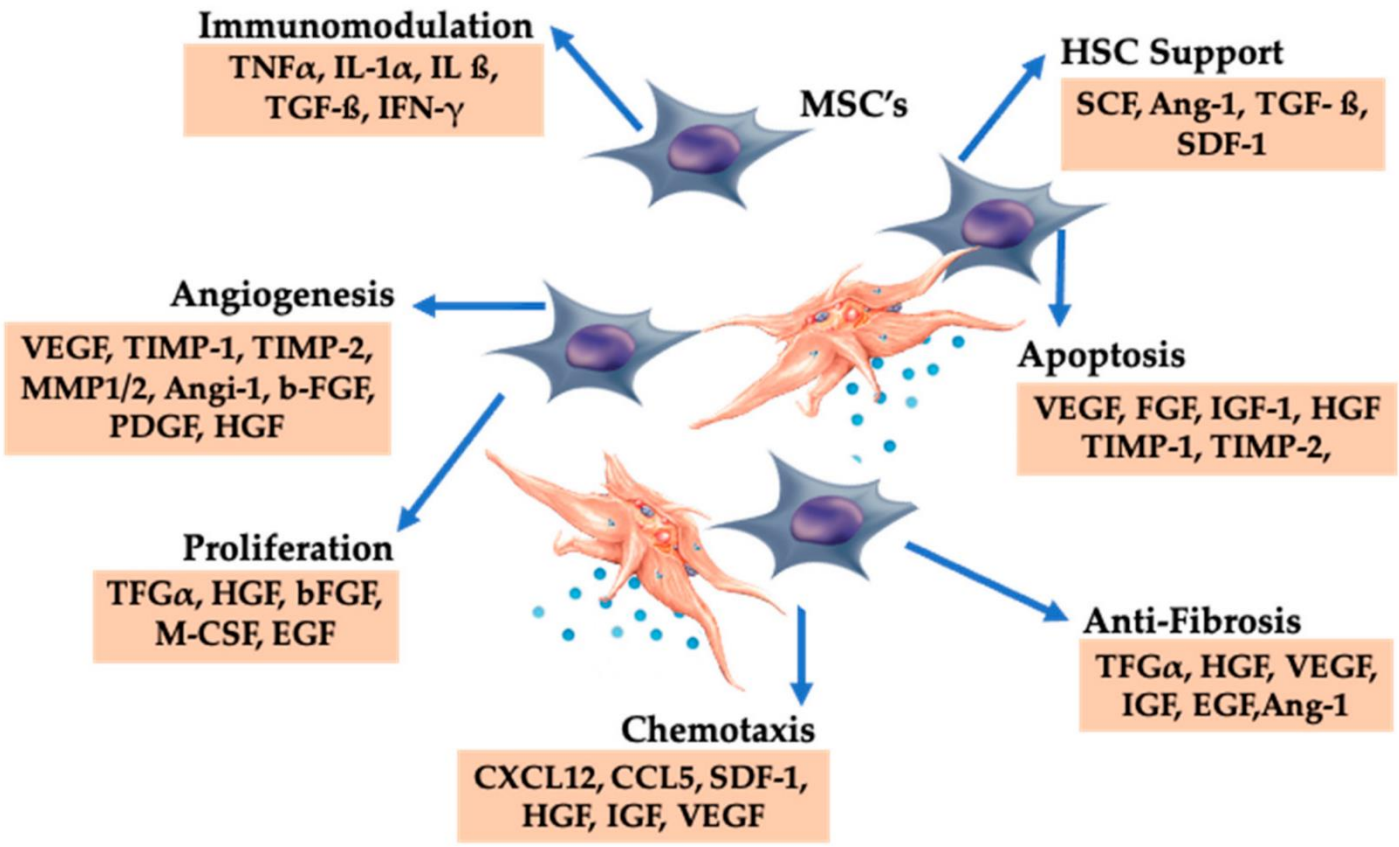


Adapted and Modified from Everts et al. and Herr et al.

- **Illustration of the multifaceted 5-HT responses following inflammatory PRP-platelet activation. After platelet activation, platelets release their granules, including 5-HT from dense granules, inciting a wide range of differential effects on various immune, endothelial, and smooth muscle cells.**

Abbreviations: SMC: smooth muscle cell, EC: endothelial cell, Treg: regular T lymphocyte, MΦ: macrophage, DC: dendritic cell, IL: interleukin, IFN-γ: interferon gamma.

FIGURE 4.



Adapted and Modified from Everts et al. and Herr et al.

- Platelet-derived growth factors and dense granular constituents are expressively involved in BMAC trophic processes, supporting MSC induced tissue repair and regeneration.

Abbreviations: MSC: mesenchymal stem cell, HSC: hematopoietic stem cell.

REFERENCES

Kang JS, Zheng Z, Choi MJ, Lee SH, Kim DY, Cho SB. The effect of CD34 + cell-containing autologous platelet-rich plasma injection on pattern hair loss: a preliminary study. J Eur Acad Dermatol Venereol. 2014; 28(1):72–79

Li ZJ, Choi HI, Choi DK, et al. Autologous platelet-rich plasma: a potential therapeutic tool for promoting hair growth. Dermatol Surg. 2012; 38(7 Pt 1):1040–1046

Eppley BL, Pietrzak WS, Blanton M. Platelet-rich plasma: a review of biology and applications in plastic surgery. Plast Reconstr Surg. 2006; 118(6):147e–159e

Mazzocca AD, McCarthy MB, Chowaniec DM, Dugdale EM, Hansen D, Cote MP, Bradley JP, Romeo AA, Arciero RA, Beitzel K (2012) The positive effects of different platelet-rich plasma methods on human muscle, bone, and tendon cells. Am J Sports Med 40:1742–1749

Browning SR, Weiser AM, Woolf N, Golish SR, San Giovanni TP, Scuderi GJ, Carballo C, Hanna LS (2012) Platelet-rich plasma increases matrix metalloproteinases in cultures of human synovial fibroblasts. J Bone Joint Surg Am 94:e1721–e1727

Freire V, Andollo N, Etxebarria J, Duran JA, Morales MC (2012) In vitro effects of three blood derivatives on human corneal epithelial cells. Invest Ophthalmol Vis Sci 53:5571–5578

Cho HS, Song IH, Park SY, Sung MC, Ahn MW, Song KE (2011) Individual variation in growth factor concentrations in platelet-rich plasma and its influence on human mesenchymal stem cells. Korean J Lab Med 31:212–218

Alsousou J, Thompson M, Hulley P, Noble A, Willett K (2009) The biology of platelet-rich plasma and its application in trauma and orthopaedic surgery: a review of the literature. J Bone Joint Surg Br 91:987–996

Pierce GF, Mustoe TA, Lingelbach J, et al. Platelet-derived growth factor and transforming growth factor-beta enhance tissue repair activities by unique mechanisms. J Cell Biol. 1989; 109(1): 429–440

Garcia BA, Smalley DM, Cho H, Shabanowitz J, Ley K, Hunt DF (2005) The platelet microparticle proteome. J Proteome Res 4:1516–1521

Everts P, Onishi K, Jayaram P, Lana JF, Mautner K. Platelet-Rich Plasma: New Performance Understandings and Therapeutic Considerations in 2020. International Journal of Molecular Sciences. 2020; 21(20):7794. https://doi.org/10.3390/ijms21207794

Magalon J, Bausset O, Serratrice N, Giraudo L, Aboudou H, Veran J, Magalon G, Dignat-Georges F, Sabatier F. Characterization and comparison of 5 platelet-rich plasma preparations in a single-donor model. Arthroscopy. 2014 May;30(5):629-38. doi: 10.1016/j.arthro.2014.02.020. PMID: 24725317.

Lei H, Gui L, Xiao R. The effect of anticoagulants on the quality and biological efficacy of platelet-rich plasma. Clin Biochem. 2009 Sep;42(13-14):1452-60. doi: 10.1016/j.clinbiochem.2009.06.012. Epub 2009 Jun 26. PMID: 19560449.

Everts PA, Malanga GA, Paul RV, Rothenberg JB, Stephens N, Mautner KR. Assessing clinical implications and perspectives of the pathophysiological effects of erythrocytes and plasma free hemoglobin in autologous biologics for use in musculoskeletal regenerative medicine therapies. A review. Regen Ther. 2019 May 10;11:56-64. doi: 10.1016/j.reth.2019.03.009. PMID: 31193111; PMCID: PMC6517793.

Kumar S, Bandyopadhyay U. Free heme toxicity and its detoxification systems in human. Toxicol Lett. 2005 Jul 4;157(3):175-88. doi: 10.1016/j.toxlet.2005.03.004. Epub 2005 Apr 7. PMID: 15917143.

Fitzpatrick J, Bulsara MK, McCrory PR, Richardson MD, Zheng MH. Analysis of platelet-rich plasma extraction variations in platelet and blood components between 4 common commercial kits. Orthop J Sports Med. 2017; 5(1):2325967116675272

Oh JH, Kim W, Park KU, Roh YH. Comparison of the cellular composition and cytokine-release kinetics of various platelet-rich plasma preparations. Am J Sports Med. 2015; 43(12):3062–3070

Fernandes D. Minimally invasive percutaneous collagen induction. Oral Maxillofac Surg Clin North Am. 2005; 17(1):51–63, vi

Magalon J, Bausset O, Serratrice N, et al. Characterization and comparison of 5 platelet-rich plasma preparations in a single-donor model. Arthroscopy. 2014; 30(5):629–638

DeLong JM, Russell RP, Mazzocca AD. Platelet-rich plasma: the PAW classification system. Arthroscopy. 2012; 28(7):998–1009

Sundman EA, Cole BJ, Fortier LA. Growth factor and catabolic cytokine concentrations are influenced by the cellular composition of platelet-rich plasma. Am J Sports Med. 2011; 39 (10):2135–2140

Beitzel K, Allen D, Apostolakos J, Russell RP, McCarthy MB, Gallo GJ, Cote MP, Mazzocca AD. US definitions, current use, and FDA stance on use of platelet-rich plasma in sports medicine. J Knee Surg. 2015 Feb;28(1):29-34. doi: 10.1055/s-0034-1390030. Epub 2014 Sep 30. PMID: 25268794.

Harm SK, Fung MK. Platelet-rich plasma injections: out of control and on the loose? Transfusion. 2015 Jul;55(7):1596-8. doi: 10.1111/trf.13160. PMID: 26172145.

Bowen RA, Remaley AT. Interferences from blood collection tube components on clinical chemistry assays. Biochem Med (Zagreb). 2014 Feb 15;24(1):31-44. doi: 10.11613/BM.2014.006. PMID: 24627713; PMCID: PMC3936985.

T, Alexander D, Barkatali B. Platelet-rich plasma: a narrative review. EFORT Open Reviews. 2021 Apr;6(4):225-235. DOI: 10.1302/2058-5241.6.200017.

Lechman, Lieberman, Kanig (1976). The theory and practice of industrial pharmacy (2nd Edition). Philadelphia, USA: Lea and Febiger

Ashok K. G. (1994). Introduction to Pharmaceutics-I (3rd Edition). New Delhi: S.K. Jain

JD, Merabishvili M, Vergauwen B, Lavigne R, Vaneechoutte M. A comparative study of different strategies for removal of endotoxins from bacteriophage preparations. J Microbiol Methods. 2017 Jan; 132:153-159. doi: 10.1016/j.mimet.2016.11.020. Epub 2016 Nov 29. PMID: 27913133.

Frautschi RS, Hashem AM, Halasa B, Cakmakoglu C, Zins JE. Current Evidence for Clinical Efficacy of Platelet Rich Plasma in Aesthetic Surgery: A Systematic Review. Aesthet Surg J. 2017 Mar 1;37(3):353-362. doi: 10.1093/asj/sjw178. PMID: 28207031.

Araki J, Jona M, Eto H, et al. Optimized preparation method of platelet-concentrated plasma and noncoagulating platelet-derived factor concentrates: maximization of platelet concentration and removal of fibrinogen. Tissue Eng Part C Methods. 2012;18(3):176–185.

Fukaya M, Ito A. A New Economic Method for Preparing Platelet-rich Plasma. Plast Reconstr Surg Glob Open. 2014;2(6): e162.

Wahlström O, Linder C, Kalén A, Magnusson P. Variation of pH in lysed platelet concentrates influence proliferation and alkaline phosphatase activity in human osteoblast-like cells. Platelets. 2007;18(2):113-118.

Lei, H., Gui, L., & Xiao, R. (2009). The effect of anticoagulants on the quality and biological efficacy of platelet-rich plasma. Clinical biochemistry, 42(13-14), 1452-1460. https://doi.org/10.1016/j.clinbiochem.2009.06.012

Giraldo, C. E., Álvarez, M. E., & Carmona, J. U. (2015). Effects of sodium citrate and acid citrate dextrose solutions on cell counts and growth factor release from equine pure-platelet rich plasma and pure-platelet rich gel. BMC veterinary research, 11, 60. https://doi.org/10.1186/s12917-015-0370-4

Singh S. (2018). Comparative (Quantitative and Qualitative) Analysis of Three Different Reagents for Preparation of Platelet-Rich Plasma for Hair Rejuvenation. Journal of cutaneous and aesthetic surgery, 11(3), 127-131. https://doi.org/10.4103/JCAS.JCAS_108_18

Arora S, Agnihotri N. Platelet Derived Biomaterials for Therapeutic Use: Review of Technical Aspects. Indian J Hematol Blood Transfus. 2017 Jun;33(2):159-167. doi: 10.1007/s12288-016-0669-8. Epub 2016 Mar 29. Erratum in: Indian J Hematol Blood Transfus. 2017 Jun;33(2):168. PMID: 28596645; PMCID: PMC5442043.

Fadadu, P.P.; Mazzola, A.J.; Hunter, C.W.; Davis, T.T. Review of concentration yields in commercially available platelet-rich plasma (PRP) systems: A call for PRP standardization. Reg. Anesth. Pain Med. 2019, 44, 652-659.

Carofino, B., Chowaniec, D. M., McCarthy, M. B., Bradley, J. P., Delaronde, S., Beitzel, K., et al. (2012). Corticosteroids and local anesthetics decrease positive effects of platelet-rich plasma: an in vitro study on human tendon cells. Arthroscopy 28, 711–719. doi: 10.1016/j.arthro.2011.09.013

Mazzocca, A. D., McCarthy, M. B. R., Chowaniec, D. M., Cote, M. P., Romeo, A. A., Bradley, J. P., Arciero, R. A., & Beitzel, K. (2012). Platelet-Rich Plasma Differs According to Preparation Method and Human Variability. Journal of Bone and Joint Surgery, 94(4), 308-316. https://doi.org/10.2106/jbjs.k.00430

Oh JH, Kim W, Park KU, Roh YH. Comparison of the Cellular Composition and Cytokine-Release Kinetics of Various Platelet-Rich Plasma Preparations. The American Journal of Sports Medicine. 2015;43(12):3062-3070. doi:10.1177/0363546515608481

Pachito, D. V., Bagattini, N. M., de Almeida, A. M., Mendrone-Júnior, A., & Riera, R. (2020). Technical Procedures for Preparation and Administration of Platelet-Rich Plasma and Related Products: A Scoping Review. Frontiers in Cell and Developmental Biology, 8. https://doi.org/10.3389/fcell.2020.598816

Dhurat, R., & Sukesh, M. (2014). Principles and methods of preparation of platelet-rich plasma: A review and author's perspective. Journal of Cutaneous and Aesthetic Surgery, 7(4), 189. https://doi.org/10.4103/0974-2077.150734

Croisé, B., Paré, A., Joly, A., Louisy, A., Laure, B., & Goga, D. (2020). Optimized centrifugation preparation of the platelet rich plasma: Literature review. Journal of Stomatology, Oral and Maxillofacial Surgery, 121(2), 150–154. https://doi.org/10.1016/j.jormas.2019.07.001

Alhumaidan, H., Cheves, T., Holme, S., & Sweeney, J. D. (2011). Manufacture of Pooled Platelets in Additive Solution and Storage in an ELX Container After an Overnight Warm Temperature Hold of Platelet-Rich Plasma. American Journal of Clinical Pathology, 136(4), 638–645. https://doi.org/10.1309/ajcpfd87thdwcsva