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Advantages and Clinical Applications of Lyophilized Platelet-Rich Plasma in Various Disease Conditions: A Review

Pradeep Mahajan[1], Ajit Kulkarni[2], Shweta Waghdhare[3], Sanskruti Mahajan[4]
[1, 2, 3, 4]*Stem Rx Bioscience Solutions Pvt. Ltd, Navi Mumbai, Maharashtra, India*

Abstract: ***Platelet rich plasma (PRP) is a biological product defined as a portion of the plasma fraction of autologous blood with a platelet concentration above the baseline. The plasma occupies 55% of blood, which is rich in immunoglobulins and proteins that have a wide range of applications in various medical fields. Plasma therapy is applied to tackle various disorders or diseases as it induces the body to develop new healthy cells. It contains important components like antibodies, coagulation factor, enzymes, fibrinogen, proteins and albumin. PRP is a unique and advanced treatment which helps to increases the body's natural healing process. Platelet lysate which is obtained from platelet rich plasma consist of various growth factors such as chemokines, cytokines, and antibacterial molecules and also has anti-inflammatory, immunomodulatory, anti-fibrotic and repairing effects. As PRP is rich in the proteins and several antibodies, it is used for various chronic therapies such as hemophilia and autoimmune disorders as well as in various severe health problems. Lyophilized Platelet-rich plasma (LPRP) therapy is currently used in various fields such as in tissue regeneration, wound healing, scar revision, skin rejuvenating effects, alopecia and for the coronavirus disease (COVID-19). It is also used to heal wounds and illnesses. LPRP therapy is gaining attraction by many health professionals as it is a safe, effective, efficient, and easy approach in procuring, preserving, and therapy. In this review we described the advantages and applications of using lyophilized PRP in various diseases which might found to be effective in different treatment.***

Keywords: Plasma, Platelet, Growth Factors, Lyophilized platelet rich plasma.

Abbreviations

EGF: Epidermal growth factor
FGF-2: Fibroblast growth factor-2
HGF: Hepatocyte growth factor
IGF -1, 2: Insulin-like growth factor-1,2
IkB: Inhibitor of Nuclear factor kappa B cells
IL: Interleukin
LPRP: Lyophilized Platelet rich plasma
NF-kB: nuclear factor kappa B cells
PDGF: Platelet-derived growth factor
PRF: platelet-rich fibrin
PRP: Platelet rich plasma
RBCs: Red blood cells
TGF β: Transforming growth factor beta
TNF-α: Tumor necrosis factor alpha
VEGF: Vascular endothelial growth factor
WBCs: White blood cells
ECM: Extracellular matrix
ARDS: Acute respiratory distress syndrome
COVID-19: Coronavirus disease-19
AGA: Androgenetic alopecia
DCL : Degenerative cartilage lesions
PRF: Platelet-rich fibrin
GF: Growth factors
POF: Premature ovarian failure

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I. INTRODUCTION

A. Platelet Biology

Platelets or thrombocytes are derived from the bone marrow. They are anucleated, colorless, discoid cellular elements with different sizes (1-3 µm size) and having 8 to 12 days life span with a density of approximately 2 µm in diameter, which is the smallest density among all blood cells [1].

The platelets count in the circulatory system varies from 150,000 to 400,000 platelets for each µL. Platelets contain a various secretory granule that are necessary for proper functioning of platelet. There are 3 kinds of granules: namely thick /dense granules, o-granules, and lysosomes. In every platelet there are around 50–80 granules. Studies propose that platelets contain abundance of growth factors and cytokines that can influence inflammation, angiogenesis, and stem cell proliferation and migration [2].

B. History

The concept and description of PRP began in the field of hematology [3]. Hematologists coined the term PRP during 1970s to depict the plasma with a high platelet count then peripheral blood, which was initially tried as a transfusion product to treat thrombocytopenia patients [4]. Platelet-rich plasma (PRP) also knows as platelet rich growth factors (GFs), platelet-rich fibrin (PRF) matrix, PRF, and platelet concentrate [5]. PRP has been extensively used in sports injuries in the musculoskeletal field [6]. PRP is now used in other medical fields such as cardiac surgery, pediatric surgery, gynecology, urology, plastic surgery, and ophthalmology [7].

C. Platelet-Rich Plasma (PRP)

PRP is a biological product consisting of the plasma fraction portion of autologous blood having a platelet concentration 3 to 5 times higher than physiologic concentration of thrombocytes in whole blood [8]. PRP was first introduced by Whitman *et al*., to the oral surgery community and since then it has been widely used in dentistry and in other medicine branches [1].

PRP consists of the high concentration of growth factors and cytokines. These growth factors includes platelet derived growth factor, insulin-like growth factor, vascular endothelial growth factor, epidermal GF, hepatocyte GF, platelet-derived angiogenic factor, matrix metalloproteinases 2, 9, and insulin-like GF 1, 2 (IGF-1, IGF-2), interleukin 8, transforming growth factor beta, fibroblast growth factor, epidermal growth factor, connective tissue growth factor, and interleukin-8 [9,10]. Platelet activation triggers the release of these growth factors by a variety of substances or stimuli such as thrombin, calcium chloride, and collagen [11]. PRP as a regenerative medicine contains the biomolecules present in both platelets and plasma which are capable of modulating the altered biological processes. PRP has immunomodulatory, anti-inflammatory, angiogenic, anti-fibrotic and anti-apoptotic properties. It can also stimulate different cells and has no general or adverse effects on other tissues [12].

Fig.1 Component of Platelet Rich Plasma

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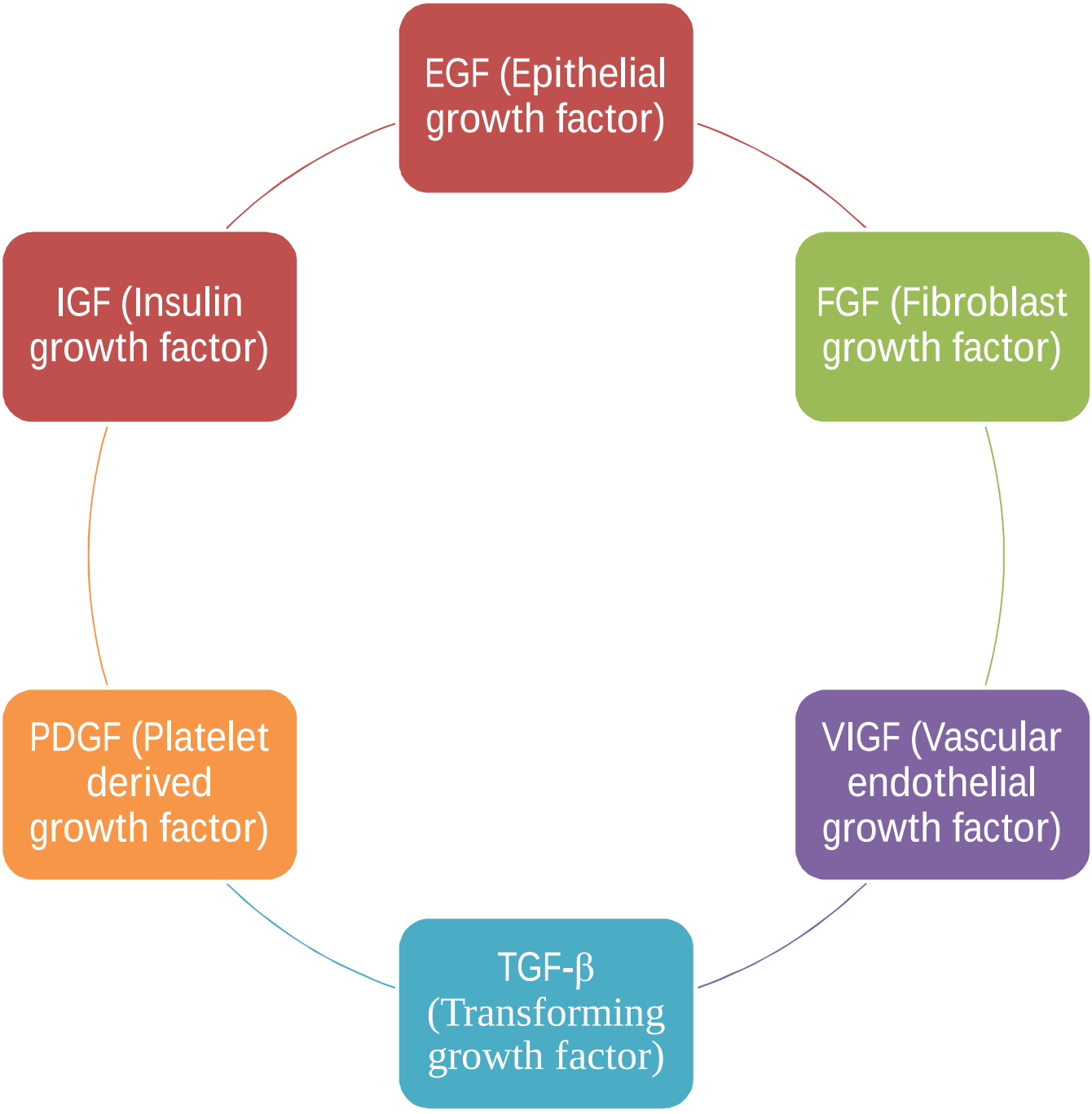

Fig. 2 Platelet Rich Plasma Growth Factor

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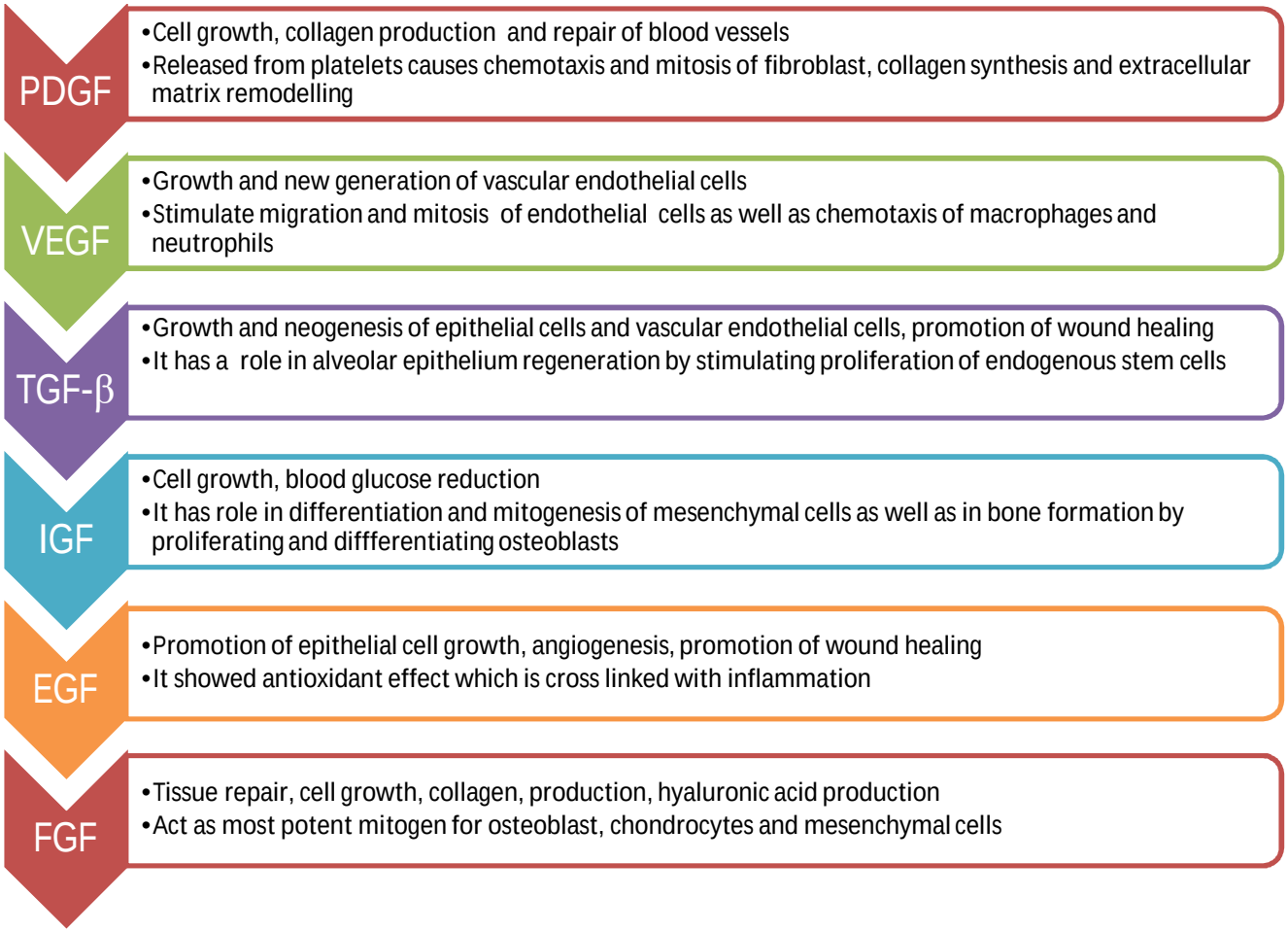

Fig. 3 Growth Factors & their Functions

D. Mechanism of Action of PRP

The action of PRP is derived by the degranulation of cellular alfa-granules, consisting of growth factors and cytokines. These biochemical messengers generate during the clotting process while the coagulation occurs. It initiates with secretion of the growth factors, within first hour of the process of clotting, with majority of the messengers derived in 1-2 hours. Hence, PRP must be synthesized in an anticoagulated state and used within ten mins of clot initiation. The anticoagulated state PRP remains viable for up to 8 hours in a sterile environment. The growth factors in the granules within the platelets are in an inactive state and made soluble by initiation of the clotting mechanism [13].

Following an initial burst of PRP-GFs, the synthesis and secretion of the additional GFs by the platelets continue for the remaining lifespan of 5-7 days. After this, the inflammatory macrophages continue to stimulate healing by secretion of similar growth factors. Therefore, the number of platelets in the blood clot within the graft, wound, or adherent to a flap sets the rate of wound healing. PRP being a rich source of platelets serves as the supplement source, during the physiological course of healing, and provides an increased concentration of GF. This promotes the cellular activity and boosts the healing procedure [13].

E. PRP in lyophilized form (LPRP)

The use of platelet rich plasma in a lyophilized form has a several advantages such as their consistency in various growth factors concentration which helps to eliminate the variability among existing PRP formulations, no depletion of growth factors, availability in large quantities, anti-bacterial activity remains unchanged, very few or no adverse effects as it devoid of WBCs and RBCs, found to be cost effective, also helps to increased stability of products [1].

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F. Applications

Platelet rich plasma is another approach in tissue regeneration which has been widely used in various surgical fields including dental surgical procedures. Recently, PRP has become a valuable adjunct to promote healing in many procedures in dental and oral surgery including ablative surgical procedures, and surgical repair of the alveolar cleft and periodontal plastic surgery, as well as procedures relating to the placement of osseointegrated implants. "In such procedures, the adhesive nature of PRP facilitates the easier handling of graft material, with more predictable flap adaptation and hemostasis, and a more predictable seal than is the case with primary closure alone" [13].

G. PRP in Gynecological Disorder

Hua *et al.*, observed a shorter tissue healing time, milder adverse effects and complete cure (93.7%) in PRP treated patients than laser treatment patients (92.4%). They reported PRP as a promising treatment in these patients [14]. Behnia-Willison *et al*., reported PRP treatment in patients resulted in clinical improvement in size of LS lesion and complete recovery in 28.6% in these patients. They also reported no complication, less pain and therefore PRP can be applied as an effective therapy for LS patients [15]. Morelli *et al.*, reported PRP treatment as an effective strategy in lowering wound infection, necrosis, faster wound healing, shorter hospital stay, reduction in post operative fever in vulvar cancer surgery patients [16].

Shirvan *et al*., reported Autologous PRP treatment found to be safe and effective in patients with vesicovaginal fistulae. PRP also prevents the need for open surgery in these patients [17]. Pantos *et al*., reported PRP treatment resulted in successful ovarian rejuvenation after 1-3 months in women with premature ovarian failure (POF) [18].

H. PRP in Viral Infection

Severe acute respiratory syndrome corona virus 2 and its variant are responsible for current COVID -19 pandemic. People who are infected by such disease can go through respiratory distress syndrome (ARDS) and cause pulmonary fibrosis in which results in progressive reduction in the patients which may lead to death in many cases. Beitia *et al.*, reported PRP as a regenerative medicine therapy which found to be effective against COVID 19 disease [1]. Karina *et al.*, found PRP treatment to be safe, effective and promising adjunctive therapy for severe covid 19 patients in phase I/II clinical trial [19].

I. PRP in Rheumatoid Arthritis

Rheumatoid arthritis is a chronic inflammatory joint disease that causes chronic inflammation in joints manifesting with swelling, pain, synovitis and joint destruction [20].

Badsha *et al*., reported PRP treatment resulted in improvement in joint inflammation, found to be safe and useful therapy in patients with rheumatoid arthritis who do not respond to established treatment [21].

Evija *et al.*, reported PRP treatment as an effective against RA patients. It helps to improves regeneration of damaged tissue by enhancing formation of new vessels and also helps in delivering VEGF (Vascular endothelial growth factor which promotes in formation of blood vessels that increases vascularity in injured tissue. They found PRP as a simple, cost effective and easiest method that has been advised for the treatment of patient suffering from knee disorder [22]. Tong *et al*., administrated PRP in human rheumatoid FLS cells and found that it effectively altered the production of inflammatory mediators and enhances neo vascularization, which thought to be accelerated the healing process. It helps to regulate factors in p13/AKT pathway, which involved in pathogenesis of disease. Platelet rich plasma serves as novel therapeutic treatment which helps to minimize the destructive progression of rheumatoid arthritis [23].

J. PRP in Lung Injury

Shibani *et al.*, reported reduction in pulmonary injury, inflammation and vascular leak. LPRP treatment also found to be effective on the vascular endothelium and in lung function following hemorrhagic shock [24]. In Acute respiratory distress syndrome (ARDS), a life-threatening lung injury, fluid leaks into the lungs that cause pulmonary edema which leads to difficulty in breathing. The current treatment includes mechanical ventilation, fluid management, use of a single anti-permeability factor, such as activated protein C have limited success. PRP contains angiopoietin (Ang) 1 and other various other growth factors such as platelet-derived growth factor, which helps in stabilizing vascular integrity. PRP, through Ang-Tie2 signaling, can also prevents endotoxin-induced pulmonary edema [25].

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K. PRP in Osteoarthritic Condition

Haleem *et al*., found PRP as a good facilitator and carrier for cartilage tissue engineering as it reduces inflammation and showed improvement in cartilage repair. In osteochondral defects, PRP helps in stem cells differentiation to cartilage and bone [26]

Kon *et al*., first reported PRP treatment on patient with osteoarthritic knees with significant improvement, reducing pain and symptoms, giving better results and recovering articular function. PRP treatment in younger patient and in milder cases is more effective and long lasting [27]. Sanchez *et al*., reported PRP treatment in articular cartilage in soccer players improve symptoms and accelerate healing in nontraumatic avulsion fractures [28]. PRP injection is found to be safe and effective in reducing pain and improve knee function in degenerative cartilage lesions (DCL) patients [29].

L. PRP in Wound healing and Sports Injury

In musculoskeletal lesions, the mechanism of tissue repair is often slow and incomplete that highly impacts the work and life of professional athletes' patients. Fast recovery with full efficiency is up most important in these patients. The current available treatment is insufficient for full recovery. PRP is a safe and attractive biological approach to enhance reparative process in tissues. PRP can be effective in the treatment of ligamentous, tendinous, muscular and cartilaginous injuries [30]. PRP because of its biological properties such as local delivery of autologous bioactive agents influence important physiological mechanisms such as inflammation, angiogenesis, or extracellular matrix synthesis and helps in tissue repair. Because of its safety, biocompatibility and healing properties of PRP, it is widely used in athletes to treat tendon and muscle injuries [31]. PRP can be used to treat ligament and muscle strains, lateral epicondylitis, anterior cruciate ligament, tears of the rotator cuff, and Achilles tendon. It can also be used safely during surgery at the site of injury [32].

M. PRP in Skin Disorder

Platelet-rich plasma because of its characteristic properties has received a considerable attention in the dermatology field. PRP alone or in combination with other therapies has been effective in some cosmetic problems and skin diseases. PRP in skin aging problems such as pigmentation, wrinkles, loose skin and coarseness induced extracellular matrix (ECM) remodeling resulting in increased expression of matrix metalloproteinases which removes photodamaged ECM components and stimulates dermal fibroblast proliferation and collagen synthesis [33]. Everts *et al*., and Cameli *et al* reported a decrease in brown spot count and area, mean wrinkle count and volume, SLEB thickness, skin redness, increase in SLEB density, Skin gross, elasticity, barrier function, capacitance, smoothness, scaliness and kurtosis numeric indexes in PRP treated cases [34,35]. PRP treatment can improve overall facial and cheek appearance [36] and vitiligo [37, 38].

N. PRP in Alopecia

Androgenetic alopecia (AGA), a common age-related hair loss disorder, has limited therapy and no cure. Both men and women are affected with this disorder in their lifetime. Hair restoration is an option and has high demand. Platelet-rich plasma (PRP), as a regenerative treatment has been recently explored as a treatment for AGA and other dermatological conditions like acne scars, wound healing etc. Growth factors present in PRP can bind with their receptors expressed on stem cells of hair follicle and associated tissues and proliferate which leads to stimulation of hair regrowth [39- 43]. Anitua *et al*., [44] reported a satisfied overall clinical improvement after PRP treatment in AGA patients after 1 year of follow-up. They reported that all measures such as hair diameter, mean hair density, epidermal thickness, terminal/vellus hair ratio, terminal/miniaturized hair ratio and perifollicular neoangiogenesis were significantly improved as well as a significant decrease in perivascular inflammatory infiltrates. Alves and Grimalt [45] in a randomized, blinded study showed a significant improvement in mean anagen and mean telogen hairs as well as total hair density in PRP treated patient group as compared to placebo group. Similar results were also reported by Gentile *et al*., [46] and Cervelli *et al*. [43].

II. CONCLUSION

The role of platelets and its secretary biomolecules consisting various growth factors, chemokines, cytokines, antibacterial molecules etc are well known and applied in various medical fields. Platelet-rich plasma therapy is a simple, economical, and feasible in orthopedics, surgery, ophthalmology. Although experience and clinical data, dose are important factors to be optimized for such therapies. Additionally, LPRP therapy is implemented to tackle skin disorder, lung injury, wound healing, sport injury, and also for COVID-19.

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LPRP has been found to be effective in reducing complications and improving health of COVID-19 patients. Even after having many reported positive effects of using PRP, many studies required to proves its safety and standardization of PRP. In this review we described the advantages and applications of using lyophilized PRP in various diseases which might found to be effective in different treatment. However, further studies are required to prove its efficacy.

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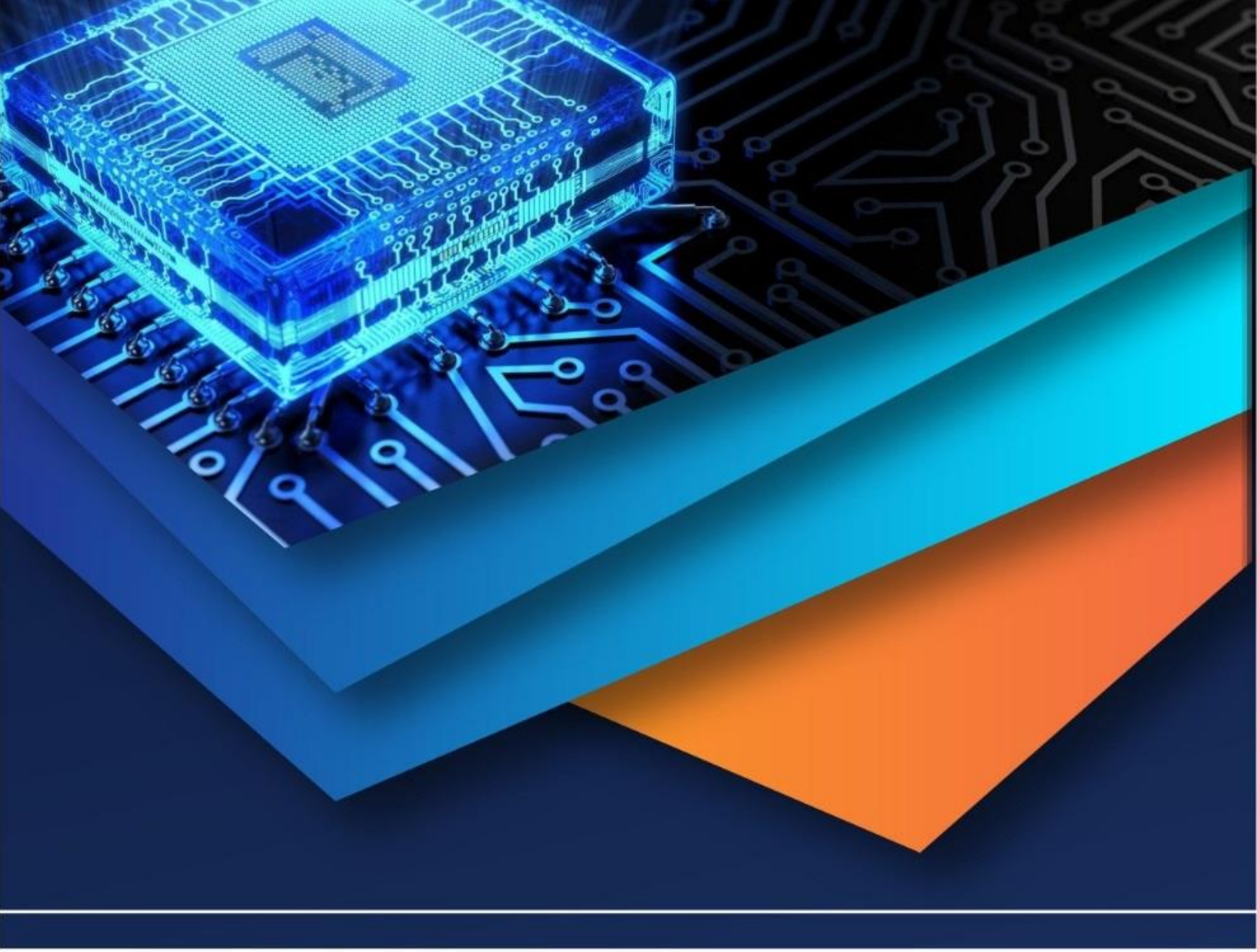

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