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REVIEW ARTICLE

Application of Platelet-Rich Plasma as a Stem Cell Treatment - an Attempt to Clarify a Common Public Misconception

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Abstract: In recent years, there has been a significant increase in the practice of regenerative medicine by health practitioners and direct-to-consumer businesses globally. Among different tools of regenerative medicine, platelet-rich plasma (PRP) and stem cell-based therapies have received considerable attention. The use of PRP, in particular, has gained popularity due to its easy access, simple processing techniques, and regenerative potential. However, it is important to address a common misconception amongst the general public equating to PRP and stem cells due to the demonstrated efficacy of PRP in treating musculoskeletal and dermatological disorders. Notably, PRP promotes regeneration by providing growth factors or other paracrine factors only. Therefore, it cannot replenish or replace the lost cells in conditions where a large number of cells are required to regenerate tissues and/or organs. In such cases, cell-based therapies are the preferred option. Additionally, other tools of regenerative medicine, such as bioprinting, organoids, and mechanobiology also rely on stem cells for their success. Hence, healthcare and commercial entities offering direct-to-customer regenerative therapies should not mislead the public by claiming that the application of PRP is a stem cell-based therapy. Furthermore, it is important for regulatory bodies to strictly monitor these profit-driven entities to prevent them from providing unregulated regenerative treatments and services that claim a broad variety of benefits with little proof of efficacy, safety concerns, and obscure scientific justification.

Keywords: Adult stem cells, angiogenesis, bioprinting, embryonic stem cells, growth factors, induced pluripotent stem cells, mesenchymal stem cells, regeneration.

1. INTRODUCTION

Regenerative medicine has been practiced in a variety of settings for decades. Human regenerative medicine is a multidisciplinary branch of medicine that aims at replacing degenerated cells or regenerating new human cells, tissues, or organs that have been lost or damaged due to the effects of aging, illness, or congenital defects. The main objective of regenerative medicines is to restore normal functions of the cells or tissues in patients affected by degenerative disorders or conditions. A variety of methods, including tissue engineering, cellular treatments, medical devices, and artificial organs, are being utilized to achieve this target. Platelet-rich plasma (PRP), autologous mesenchymal stem cells (MSCs), and several other allogenic biologics are some of the currently available treatment regimens [1, 2].

In humans, regeneration refers to the reconstruction of damaged tissues or organs resulting from an injury. This contrasts with wound healing, also known as partial regeneration, which includes sealing up the damaged site with a gradation of scar tissue to close the injury site completely. Some tissues, such as skin, the vas deferens, and major organs, such as the liver, have considerable ability to regenerate, while others, such as bone, are believed to have little or no capability to regenerate after injury.

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Nowadays, regenerative therapies are being used to treat hip pain, torn or strained ligaments, rheumatoid arthritis inflammation, osteoarthritis degraded cartilage, discomfort from bulging vertebra discs, degenerative neurologic diseases, and back pain caused by lumbar degenerative disc disease [1, 3-4]. Among different tools of regenerative medicine, PRP and stem cell-based therapy are very popular. However, there is a lack of proper explanation and knowledge regarding these two treatment modalities among patients or the general public, leading to some misconceptions about their healing potential. While both treatments have shown potential in the treatment of musculoskeletal and dermatological complications, they differ significantly in terms of their biological compositions, features and modes of action. Hence, this article aims to clarify these differences and dispel existing misconceptions to prevent the exploitation of patients by commercial healthcare entities.

2. TOOLS OF REGENERATIVE MEDICINE

Regenerative medicine is a very promising field for the development and use of novel bioengineering technology. To address the inherent biological and molecular complexity, multi-scale organization, and spatiotemporal features of regeneration processes, a variety of technological methods may be employed. The collaborative technique is producing more integrated therapeutic methods, which are altering the traditional view of implants, gadgets, medications, or biomaterials [5]. Regenerative concepts such as stem cell-based treatments, PRP therapy, prolotherapy, and tissue engineering are among the most ambitious. Several complementary areas of bioengineering, such as mechanobiology, biomaterials, cellular delivery, sensing and imaging as well as computational and mathematical modeling, are becoming more essential tools of regenerative medicine (Fig. **1**) [5-6].

Currently available therapeutic techniques that use allogenic cells or other tissue components from a donor may result in complications for the patients, including immunologic rejection. In comparison, autologous cell therapy, which uses cells from the same individual, is less likely to be rejected, making it a safer type of treatment. Autologous cell therapy is tailored to the individual requirements of the patient and takes into consideration any complications that may occur during treatment. A variety of effects may be achieved by incorporating foreign genes or unique structures into nucleic acids and nucleic acid analogs that are taken up by target cells. These effects include regulating protein expression, substituting a missing gene, and targeting particular genes and proteins [7-8]. Molecular treatment is the use of small or large molecules (oligonucleotides, proteins, or enzymes) that interact with the patient's endogenous genetic material, transcription, or translation, restoring the endogenous cellular process that results in the creation of the patient's own product [9].

Currently, four major regeneration methods are available: stem cell treatment, PRP therapy, lipogems, and prolotherapy, as depicted in Figure **1**. Besides, three-dimensional (3D) bioprinting, mechanobiology, and organoid cultures are considered advanced fields of regenerative medicine. Stem cell therapy is the most well-known of these. There have been many studies that have shown promising outcomes with the use of stem cells and PRP for the healing of tendons, osteoarthritis, and the intervertebral disc. During an injury, platelets, the small components of blood that are rich in growth factors (GFs) play a critical part in the formation of blood clots, which is crucial for healing. It is a well-known fact that the concentrations of platelets in injured tissues are critical in the healing of wounds. Platelet-rich plasma is composed of blood components, more specifically, the plasma and concentrated platelets. It works by nourishing cells that have the ability to mend on their own, or it may assist in the healing process by resolving damaged tissues, depending on the situation. It is more frequently used in the regeneration and rebuilding of skeletal and connective tissues in the treatment of periodontal and maxillofacial disorders, as well as in the treatment of sports-related injuries [10-11].

Stem cells are primordial cells that may be acquired either from embryos or from adult tissues rather than from blood. Stem cells have the ability to self-renew and can differentiate into various distinct kinds of cells in the adult body, depending on the environment or culture medium. Beyond these characteristics, stem cells also generate several GFs and cytokines that aid in the repair of wounds and other tissue damage [12-13]. Furthermore, stem cell therapy is more effective in the treatment of numerous degenerative and inflammatory diseases where replacing the destroyed cells in tissues or organs is the only choice [14].

Lipogems treatment makes use of micro-fractured adipose tissue, which includes a diverse population of cells, including mesenchymal stem cells (MSCs) that are injected into the patient. To obtain cells for this therapy, adipose tissue is exposed to moderate mechanical stresses [15]. Prolotherapy, another type of regenerative therapy, includes a nonsurgical regenerative injection technique. This therapy uses small amounts of an irritant solution to promote the growth of normal cells and tissues at the site of painful and degenerated tendon insertions, joints, ligaments, and adjacent joint spaces over a period of time. Typically it requires several treatment sessions [16-17]. Irritant solutions are often composed of dextrose (d-glucose), a form of glucose that occurs naturally in the body, but they may also include other ingredients such as polidocanol, manganese, zinc, human growth hormone, pumice, ozone, glycerin, or phenol, among others [18].

Three-dimensional (3-D) bioprinting is merging living cells with biomaterials to create complex, multidimensional tissues via controlled layer-by-layer deposition of cells and/or bioink with hierarchical structural characteristics and prolonged cellular viability in 3-D space. Tissue engineering, synthetic biology, micro/nanofabrication, and bioprocessing biomaterial manufacture all assisted 3-D bioprinting. This approach is multidisciplinary, and to move bioprinting-based tissue engineering beyond the laboratory, engineers, scien-

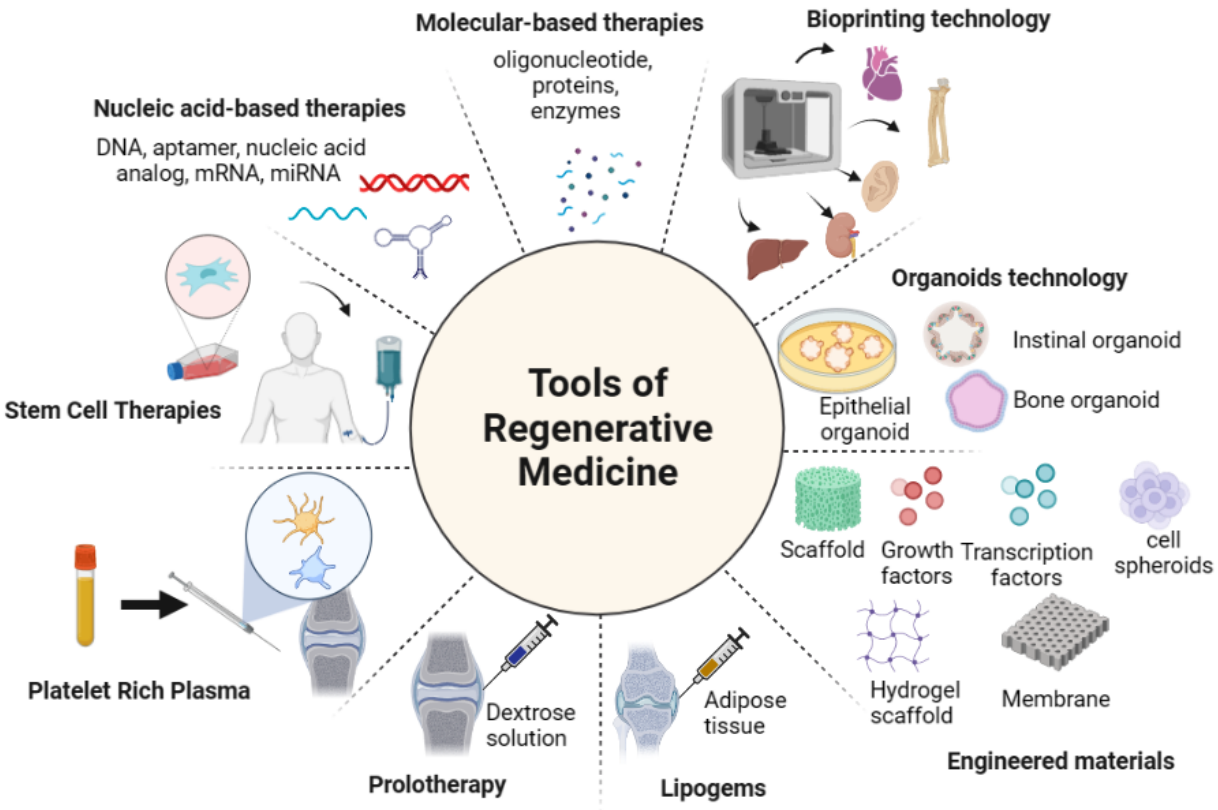

Fig. (1). Tools of regenerative medicine [6-8, 27-29]. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

tists, and clinicians must conduct comprehensive and systematic studies on bioink optimization, bioreactor engineering, and cell culture environment to enable high-throughput production that is associated with efficient screening assays. Because the structure of biological tissues and organs is so complex, it is essential to develop printing equipment capable of printing hybrid materials (bioinks) with high resolution, speed, biocompatibility, and repeatability. This may be done by integrating bioprinting technologies with other enabling techniques such as 3-D cell culture, bioreactor technology, microfluidics, and organ-on-a-chip. With appropriate bioinks and sophisticated biofabrication methods, this technology will be able to bridge the currently enormous gap between the lab and the fab, eventually meeting current clinical and industrial requirements and pushing the limits for enhanced drug discovery and regenerative medicine [19-20].

Mechanobiology is becoming more relevant in tissue regeneration and therapy design. This understanding is assisting in the development of more precise and effective biomaterials and scaffolds. Scaffolds are important in tissue engineering techniques because they offer an architecturally appropriate environment for cells to develop in. A scaffold should be able to accommodate the cells of interest while also providing GFs to aid in cell upkeep, proliferation, and differentiation. The scaffold should have mechanical characteristics that are appropriate for rebuilding the injured tissue. Scaffolds used in regenerative treatment should be biodegradable in the human body after the cells of interest have been delivered to the target location [21]. Initially, organic acids were used to make scaffolds, but further research discovered that the acids were poisonous to cells and harmed the microenvironment [22]. Mesenchymal stem cells grow much more rapidly on scaffolds than on plastic plates. When grown on scaffolds, their chances of survival and differentiation improve. Gingival MSCs grown on a poly (lactic acid) scaffold showed a significantly greater ability to develop into neural cell types than those produced without a scaffold and may be utilized to treat neurodegenerative disorders [23].

Organoids are 3-D multicellular *in vitro* human tissue constructions that are utilized in research to imitate their corresponding *in vivo* organs. The term ‘organoid' is relatively new, and it most often refers to constructions generated from pluripotent or adult stem cells (ASCs) from different organs [24]. Organoids generated from stem cells are not the only kind of organoid. Organoid technology is the *in vitro* creation of a 3-D structure in conjunction with stem cells to imitate the original architecture of tissues and organs, resulting in a fairly near copy of a natural structure [25-26]. Though organoid technology is being used to bridge the gaps between two-dimensional cell cultures and *in vivo* animal models, and patient-specific mimicked organ microenvironments for drug development and personalized medicine [27], it has a great prospect in the field of regenerative medicine as well. In recent

years, several researchers have reported the potential use of organoids to regenerate several organs or tissues, namely biliary epithelium, intestinal mucosa, etc.

3. PLATELET-RICH PLASMA A POPULAR THERAPEUTIC TOOL OF REGENERATIVE MEDICINE

Platelet concentrates, which most products are termed PRP one of the blood-derived products that offer interesting therapeutic perspectives. Historically, the methodological aspects and clinical applications of PRP were first described in the field of hematology [30]. Blood plasma was the only product in the early years of transfusion to treat patients with thrombocytopenia [31]. Experts in the field have the concept of PRP with various terminology since the 1970s to explain the difference in platelet count between plasma and peripheral blood [32]. They categorize platelet concentrates into four types, namely Pure Platelet-Rich Plasma (P-PRP), Leukocyte and Platelet-Rich Plasma (L-PRP), Pure Platelet-Rich Fibrin (P-PRF), and Leukocyte- and Platelet-Rich Fibrin (L-PRF), by which P-PRP and L-PRP represent the inactivated liquid form of these products while P-PRP gels and L-PRP gels are named to distinguished the activated versions of PRP. Owing to its adherence and homeostatic properties, PRP was also exploited in maxillofacial surgery as PRF some years later, and with its anti-inflammatory characteristics, it evidenced accelerated cell proliferation [33]. Hitherto, PRP has been used predominantly in orthopedic practice to augment healing in sports-related injuries of skeletal muscle, tendons, and ligaments [34]. With its growing use for the treatment of musculoskeletal injuries in sports medicine, the translational use of PRP has been progressively developed. Nowadays, PRP is clinically available in cardiac surgery, pediatric surgery, gynecology, urology, plastic surgery, and ophthalmology [35].

Dermatology is currently one of the emerging fields of study that uses PRP as an ever-expanding treatment modality, particularly in tissue regeneration [36], scar revision [37], wound healing [38-39], skin rejuvenating effects [40], and alopecia [41]. From a biochemical standpoint, the proinflammatory-rich environment of the wound in chronic ulcers diminishes the integrity of the extracellular matrix, which impairs healing [42]. High protease activity had an impact on the concentration of protein GFs while PRP replenishes the source of GFs and consequently, activates their mitogenic, angiogenic, and chemotactic properties [43]. In aesthetic dermatology, PRP demonstrated an ability to enhance dermal elasticity by collagen production as well as keratinocyte and fibroblast proliferation [44]. Platelet-rich plasma also promotes hyaluronic acid synthesis to rejuvenate the aging skin through various GFs and cell adhesion molecules [45-46]. Hyaluronic acid was found to improve skin moisturization through binding and retaining the capacity of water molecules leading to swelling that gives volume and skin turgor. Thicker collagen was observed in those who received PRP injection, which was explained by simulation of collagen I and matrix metalloproteinases 1 and 2 (MMP1 and MMP2) [47]. Platelet-rich plasma use for skin rejuvenation explicitly has gained the interest of dermatologists in the cosmetic industry. Generally, such a combination of human plasma components with the multiple secretome and GFs from human platelets continues to evolve as an important treatment modality in different medical fields with many applications in soft and hard tissue regeneration. The readiness of PRP in clinical settings can be attributed to its standardization and simple preparation, safety, GF release and cost-efficiency, that is better than traditional recombinant GFs or synthetic biomaterials.

4. ISOLATION OF PLATELET-RICH PLASMA

Platelets or platelet-derived GFs can be obtained from whole blood or platelet apheresis using either autologous or allogeneic preparation [48]. These two methods isolate distinct amounts of platelets, red blood cells (RBCs), white blood cells (WBCs), and plasma, depending on the type of device chosen as well [49]. The clinical application of autologous PRP is largely debatable due to the controversial issue of inconsistent PRP quality among patients [50-51]. Nevertheless, autologous PRP is still preferred for some clinical practices based on various reasons [38, 49].

There are two ways of preparing PRP, either open or closed techniques on the principle of centrifugation [30]. The former exposes the product to the environment of the working area and equipment (i.e., pipettes or collection tubes) thus proper microbiological handling should be applied to avoid the risk of product contamination. Since PRP is isolated through the process of differential centrifugation, an appropriate closed technique is preferred using regional-certified commercial medical devices that process the product in a closed (unexposed) system [52-53]. There are various systems used to generate PRP in a reproducible manner. In general, a small volume of drawn blood collected by venipuncture is kept in a tube that contains an anticoagulant (usually with acid citrate dextrose or sodium citrate solution). Depending on the device used, the blood is then subjected to either single- or double-spin centrifugation. An adjusted acceleration force facilitates the sedimentation of certain cellular constituents which vary significantly based on specific gravity [30]. Basically, the default settings of the centrifuge to obtain PRP at an adjustable concentration are readily established by the manufacturer and it should be done according to the manufacturer's instructions. Upon centrifugation, the tube will produce 3 basic layers (Fig. **2**): the first layer at the top contains the platelet-poor plasma (PPP) followed by the middle layer where the PRP is contained and the bottom layer which consists of the red blood cells and leukocytes sediments. Platelet-rich plasma is obtained upon the removal of PPP [54]. Activation of the platelets can either be done before or after the application of the PRP [38]. In fact, there is not enough evidence to support the significance of the platelet activation steps or which agonist is prefe-

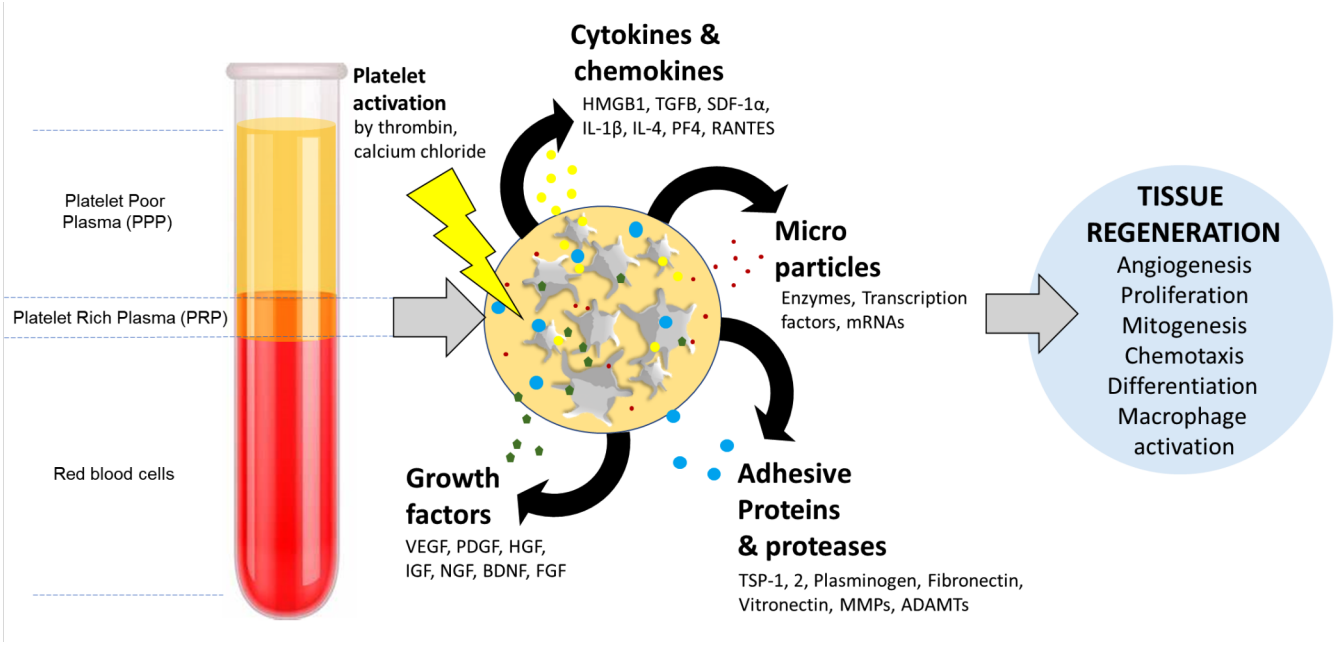

Fig. (2). Isolation of Platelet-Rich Plasma (PRP) and biological mediators from activated platelets involved in tissue regeneration. The blood collecting tube containing an anticoagulant will produce 3 basic layers upon centrifugation: the first layer at the top contains the platelet-poor plasma (PPP); the middle layer where the PRP is collected; the bottom layer which consists of the red blood cells and leukocytes sediments. Activated platelets, releasing various GFs, cytokines, adhesion molecules, and microparticle containing mRNA, regulate various cellular interactions to stimulate tissue repair in tissue regeneration Abbreviations: HMGB1: High mobility group box protein 1, TGFB: transforming growth factor beta, SDF-1: stromal cell-derived factor 1 alpha, IL-β1: interleukin-β1, IL-4: interleukin-4, PF4: platelet factor 4, RANTES: Regulated upon Activation Normal T Cell Expressed, mRNA: messenger RNA, TSP-1: thrombospondin-1, MMPs: matrix metalloproteinases, ADAMTs: A disintegrin and metalloprotease with thrombospondin motifs, VEGF: vascular endothelial growth factor, PDGF: platelet-derived growth factor, HGF: hepatocyte growth factor, IGF: insulin-like growth factor, NGF: nerve growth factor, BDNF: brain-derived neurotrophic factor, FGF: fibroblast growth factor. Redrawn and adapted from Sánchez et al. 70. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

rred. Aggregation inducers like thrombin and calcium chloride are commonly used to activate platelets and stimulate degranulation to release GFs [55]. However, some researchers claimed that the application of inactivated platelets to the affected organ or tissue followed by *in situ* activation demonstrated better results [56]. In this method, the use of such aggregators is not required since the platelets are automatically released during the administration to exert their function.

5. GENERAL FEATURES OF PLATELET-RICH PLASMA

Human plasma and platelets are crucial components of PRP as a regenerative biomedicinal approach. The plasma constitutes the intravascular fluid part of our extracellular fluid (ECF) with up to 95% of it being water by volume [30]. Also, it incorporates glucose, dissolved proteins (6–8%), hormones, and several other biologically active molecules, including GFs such as hepatocyte growth factor (HGF) and insulin-like growth factor-1 (IGF-1) [57]. Most importantly, human plasma carries clotting factors called fibrinogens that form a matrix of fibrin scaffold to entrap proteins and GFs and to function as a carrier for the host autologous cells, including the MSCs [30]. Platelets, on the other hand, store and deliver other biologically active molecules to exert their regenerative function.

Human platelets, also called thrombocytes, are tiny nucleated discoid cells of approximately 2 µm in diameter with a life span of 7-10 days [58]. Platelets are comprised of many secretory granules mainly from these three different types namely: dense granules, lysosomes, and α-granule, with the latter being the major storage granule of platelets. It is derived from megakaryocytes in the bone marrow. Megakaryocytes differentially sort and package α-granule contents into long beaded-proplatelet extensions that regulate its production [59]. Platelets promote coagulation during a vascular lesion through the release of the GFs from these granules after its activation. There are approximately 50–80 α-granule in each platelet that is responsible for the homeostasis processes, which are adhesion, activation, and aggregation.

Besides, platelets also exert other important biological roles in immune response, inflammation, angiogenesis, and tissue regeneration and remodeling [40]. Tissue regeneration is mediated via the recruitment of progenitor cells to the sites of injury. Interestingly, some findings showed that platelets are partly contributed to the maintenance of brain homeostasis and its cell interaction via microparticle release, exosome delivery,

and receptor interactions at local sites. Platelets act as a reservoir for GFs, cytokines, and other biological mediators, including PDGF, PF4, EGF, VEGF, BDNF, TGF-ß, and HGF among others, that release once platelets are activated and aggregation is initiated. Interestingly, platelets contain both pro- and antiangiogenic regulatory proteins in separate α-granule that undergo differential release once activated [59]. Upon activation, platelets also release the sub-micrometer diameter vesicles, called exosomes, into the local milieu. It contains mRNA that is involved in vascular remodeling, in the dose-dependent effect on migration and osteogenic differentiation of MSCs, and in the regulation of neural precursor cells in the neurogenic niches [60].

6. MECHANISM OF ACTION OF PRP IN REGENERATION

Despite various potential clinical benefits of platelets, the mechanisms governing PRP-mediated tissue regeneration are still poorly understood. Generally, PRP delivers a set of complex mechanisms in precision regenerative medicine tissue regeneration. Clinical PRP (C-PRP) product technologies continue to develop as a biological treatment modality that works through the delivery of biomolecules carried by high concentrations of platelets upon activation at the target tissue sites [61]. It is well documented that PRP releases cytokines, chemokines, and GFs such as HGF and SDF-1 that modulate recruitment, proliferation, and activation of neutrophils, fibroblasts, monocytes, MSCs, SMCs, and other cell types mainly involved in tissue repair and wound healing [39]. These GFs and the bioactive molecules present in PRP stimulate 4 main actions at the site of administration, such as proliferation, migration, cell differentiation, and angiogenesis leading to various immunomodulatory and inflammatory cascading events that promote healing and tissue repair.

Angiogenesis is a highly ordered process that involves the sprouting, splitting, and organization of microvessels from pre-existing blood vessels formed in the earlier stage of vasculogenesis [62]. Angiogenesis outgrows pre-existing blood vessels to enhance intrinsic tissue regeneration by restoring the blood flow, allowing the delivery of oxygen and nutrients, and facilitating the removal of byproducts from the treated tissues [63]. Angiogenic activities are orchestrated by a highly coordinated interplay of both pro-angiogenic GFs and anti-angiogenic proteins and cytokines such as basic-FGF, TGF-b, VEGF, PF4, and anti-angiogenic factors [e.g., angiostatin and thrombospondin-1 [TSP-1)]. Angiogenesis is indeed a crucial mechanism that is highly regulated by PRP in damaged tissue, and the effect is both pro-angiogenic and stimulatory. It produces a precise balance of GFs and other platelet cytokines (Figure 2) for recovery of tissue function with the regulation of local inflammation, enhancement of angiogenesis, and re-epithelialization. Xu, et al. 39 showed that PRP augments skin wound healing by modulating inflammation and simulating the migration and proliferation of repair cells *in vitro.* Platelet-rich plasma treatment was found to suppress the production of immunoregulatory cytokines interleukin-17A and interleukin-1β while inducing the local vessel intensity and enhancement of re-epithelialization through secretion of VEGF and IGF-1. Interestingly, they also found that PRP treatments are effective in increasing the migration ability of ESCs, directing the differentiation of mouse ESCs into adult cells as required for healthy function.

Platelet-rich plasma triggers the recruitment of progenitor cells, including MSCs, SMCs, and endothelial and CD34-positive progenitors, promoting wound repair partly due to paracrine mechanisms. Stromal cell-derived factor-1a binds to the CXCR4 receptors on endothelial progenitor cells to induce vasculogenesis and arteriogenesis [64]. Qian et al. 65 fabricated a self-healing and injectable hydrogel containing PRP to boost diabetic wound healing. Platelet-rich plasma hydrogel significantly improved the proliferation of HUVECs *in vitro* while demonstrating accelerated angiogenesis formation *in vivo* through the sustainable release of bioactive molecules on the wound surface.

Furthermore, platelets regulate a delicate balance between cell survival and death by apoptosis, which is a typical pathological process arising in any damaged tissues [66]. It induces the release of pro-apoptotic (i.e. CD40L, Fas-L, TWEAK, TRAIL, and LIGHT) as well as antiapoptotic (i.e. SDF-1, HGF, SP-1, serotonin, and adenosine diphosphate) mediators [62]. Granzyme B (GrB) is a cytotoxic serum protease protein that participates in mediating platelet-induced apoptosis in the spleen and lung [67]. On the other hand, HMGB1, a nuclear protein that acts as a danger signal and also an important regulator of apoptosis, will be transported to the cell surface by platelets upon activation, depending on its redox status [68]. In addition, platelets also secrete microparticles that regulate apoptosis in endothelial cells (ECs) and smooth muscle cells (SMCs) while providing survival signals for several other cell types (i.e. endothelial, monocytic, and neural stem cells) [69].

7. SHORTCOMINGS OF PLATELET-RICH PLASMA

It is worth mentioning that PRP use has been widely debated for the past few decades. The literature is often contradictory, mostly related to the lack of protocol reproducibility, confused consensual terminology and classification of tested products, and poorly designed clinical experiments in terms of dose, composition, and/or therapeutic objectives [71]. Whilst various devices claim to generate the best PRP, an optimal system, taking into account the method and time of its centrifugation, plays a significant role in the capacity of a device to harvest a desired amount of concentrate platelets [72]. In fact, different devices produce different concentrations of platelets and leucocytes, and it is not feasible to assess which kit for PRP preparation works better as each preparation generates different types of PRP with different applications [71]. Therefore, neither the number of centrifugations required nor their speed duration requires standardization, provided that the therapeutic objective of the isolated PRP is specified.

Platelet-rich plasma is termed by the higher number of platelets in the volume fraction of the blood plasma, compared to the baseline serum level. It was suggested earlier that approximately 1,000 to 1,500×103/µl of platelets are required to get a therapeutic effect of PRP [73]. These levels are 3 to 5 times the physiological concentration [74]. An *in vitro* study investigating the proliferative effects of HUVECs and various platelet concentrations based on the choice of PRP preparation devices and platelet dosing strategies demonstrated that the optimal platelet dose of 1,500×103/µl is required to promote angiogenesis [63]. A recent systematic review conducted by Gentile and Garcovich 75 on both *in vitro* and *in vivo* studies indicated that a PRP concentration of 1000×103/µl of platelets is optimal for tissue repair in regenerative medicine. Excessive platelet concentrations might be less effective as the angiogenic process can be suppressed. In another study, a higher concentration was found to be unfavorable as concentrations of over 5,000×103/µl inhibited stem cell proliferation and differentiation. Meanwhile, high leukocyte levels revealed a decreased proliferation rate as the degranulation of leukocytes releases proinflammatory substances [76].

8. POTENTIAL USES OF STEM CELLS IN REGENERATIVE THERAPY

Unlike platelets, the terminally differentiated cells, stem cells are primordial cells that can be self-renewed and/or redirected to differentiate into particular cells that could be utilized to restore and repair sick or damaged tissues in humans. People with spinal cord injuries, type 1 diabetes, Parkinson's disease, amyotrophic lateral sclerosis, Alzheimer's disease, heart disease, stroke, burns, cancer, and osteoarthritis may benefit from stem cell treatments [77-78]. Unlike other cells, stem cells do not yet have a defined function and may be transformed into virtually any kind of cell that is needed. They are undifferentiated cells that can be transformed into specialized cells when the body requires them. To date, stem cell treatment is rapidly becoming a breakthrough in medicine. The capacities of stem cells are expanding with each trial, however, there are still numerous barriers to overcome. Regardless, stem cells have a huge impact on regenerative medicine and transplantology. Currently, stem cell treatment has the potential to treat untreatable neurodegenerative disorders. The use of a patient's own cells is made possible through induced pluripotency. Tissue banks are becoming increasingly popular as a source of regenerative medicine in the fight against current and future illnesses [12, 79].

When it comes to wound healing and tissue regeneration, the function of stem cells is a hot topic for researchers, with most of the focus being on stem cells' regenerative abilities. To do this, isolated stem cells must be administered directly to the location of the wound, or impregnated stem cells must be applied to bio-membranes and scaffolds to promote fast revascularization, re-epithelialization, and closure of the wound. ESCs and iPSCs are excellent candidates for this application because of their capacity to self-renew and differentiate into several lineages [80-81]. iPSCs are laboratory-created stem cells that act as a bridge between ASCs and ESCs. iPSCs are produced by introducing embryonic genes into a somatic cell (such as a skin cell), causing it to return to a "stem cell-like" state. These cells, like ESCs, are thought to be pluripotent [82]. Other types of stem cells, such as MSCs and HSCs produced from bone marrow (BM), are involved in the wound-healing process of the skin. Mesenchymal stem cells derived from the bone marrow can differentiate into a variety of cell types, such as adipocytes, osteoblasts, chondroblasts, fibroblasts, and keratinocytes. Endothelial progenitor cells (EPCs) derived from the HSC lineage, on the other hand, are important cells in the process of neovascularization [83-85]. Stem cells are precursor cells that secrete GFs and have the ability to develop into more specialized cells as they increase in number. As a result of their capacity to differentiate into cells that make up cartilage, tendons, intervertebral discs, and bone, MSCs have attracted the greatest interest in the fields of spine and orthopedics [86-87].

9. SOURCES AND FEATURES OF STEM CELLS

Researchers classify stem cells according to their ability to develop into various kinds of cells, which they call the differentiation potential, which is as follows: totipotent, pluripotent, multipotent, oligopotent, and unipotent [88]. On the other hand, based on their origin stem cells are divided into ESCs and ASCs. In contrast to ESCs, which are produced from the epiblast of the blastocyst, from which many tissues of the embryo develop, ASCs are found in adult organs, where they serve to replace damaged cells during tissue regeneration [89-90].

Throughout the human body, stem cells serve as the building blocks for every organ and tissue, stem cells may originate from a variety of different locations in the body or be produced at various times of life. There are many distinct kinds of stem cells. Among them, ESCs, which are only present during the earliest stages of development, and a variety of kinds of tissue-specific (or adult) stem cells, which emerge throughout fetal development and stay in the body throughout life as depicted in Table **1**. ASCs are also known as tissue-specific stem cells since each kind of adult stem cell generates only a small number of specialized cells that are unique to a certain tissue- epidermis, blood, and so on. Adults have tissue-specific stem cells throughout their bodies. The HSCs in bone marrow and umbilical cord blood, which produce all kinds of blood cells, are the simplest to separate and have been used in treatment for decades – as bone marrow transplants for illnesses such as leukemia, where normal blood cell production has gone wrong. Adult material from live donors or cadavers, fetal materials, and pluripotent stem cell lines have all been explored for cell therapy and regenerative medicine. Adult-derived cell material (such as MSCs or skin epithelial cells) may be collec-

Table 1. Different types of stem cells, their sources, and type of cell produced.

Stem Cell	Source	Type of Cell Produced	Refs.
Embryonic Stem Cells (ESCs)	Inner cell mass of a blastocyst, 4-5 days post fertilization.	Muscle cells, blood cells, neurons, intestinal cells, pancreatic Islet cells, and liver cells.	[96]
Adult Stem Cells (ASCs)	Hematopoietic Stem Cells	Red blood cells, white blood cells, monocytes, macrophages, natural killer cells, and platelets.	[12]
	Mesenchymal Stem Cells (bone marrow, adipose)	Osteocytes, chondrocytes, myocytes, fibroblasts, astrocytes, stromal cells, and adipocytes	[97]
	Mesenchymal Stem Cells (Dental pulp)	Chondroblast, myoblast, fibroblast, neural cell, adipocyte, endothelial cell, cementoblast, Odontoblast, and periodontal ligament cell	[98]
	Neural Stem Cells	Neuron, astrocyte, and oligodendrocyte	[99]
	Epithelial Stem Cells	Intestine, epidermis, mammary gland, and cornea.	[100]
	Skin Stem Cells	Different cell lineages of the skin	[101]
Induced Pluripotent Stem Cells (iPSCs)	Mesoderm, endoderm and ectoderm	Cardiomyocytes, adipocytes, dopaminergic neurons, motorneurons, pancreatic β-cells, hematopoietic progenitor cells, thyroid cells, and skin cells	[102]
Perinatal stem cells	Umbilical cord blood (hematopoietic stem cells) and tissue (mesenchymal stem cells)	Red blood cells, white blood cells, monocytes, macrophages, natural killer cells, and platelets. Connective and structural tissue. Osteocytes, chondrocytes, myocytes, fibroblasts, astrocytes, stromal cells, and adipocytes.	[103-106]
	Placental blood (hematopoietic stem cells) and tissue (mesenchymal stem cells)		
	Amniotic tissue and amniotic fluid (share some features with MSCs and ESCs)	adipocytes, osteocytes, neurons, myocytes, endothelial cells, and hepatic cells	[98, 107]

ted directly from patients and purified/amplified *in vitro* [91]. This kind of autologous treatment eliminates the danger of rejection. However, adult cell sources have a lower proliferation potential. This is particularly true for terminally differentiated adult cell types that rarely proliferate or organs with limited access to endogenous stem cells [86-87].

Human pluripotent stem cells (hPSCs) have been regarded as a potential cell source for regenerative therapy since their discovery. hPSCs are self-renewing and may develop into every cell type in the human body. They may be produced from supernumerary *in vitro* fertilized embryos (human embryonic stem cells or hESCs) or from adult primary cells that have been converted to pluripotency via the overexpression of a cocktail of molecules (human induced pluripotent stem cells or hiPSCs) [92-94]. hPSCs are compatible with large-scale industrial operations in GMP facilities and are as quality regulated as any other more traditional pharmaceutical product. Whereas hESCs have been utilized in the bulk of hPSC-based clinical studies so far, the field is shifting toward hiPSCs since they do not need the destruction of embryos and may therefore be used globally without limitation [95].

10. THE MAJOR CONCERN REGARDING STEM CELL-BASED THERAPY

For the time being, significant scientific and medical advances in stem cell treatment are crucial in generating new tissue for use in transplantation, and regenerative medicine must be strictly controlled for both ethical and safety concerns. Currently, stem cell application may be challenging. The first and most important step is to fully understand how stem cells function in animal models. Fear of the unknown is the biggest barrier to universal acceptance. Millions of functional, physiologically correct cooperating cells would be needed to build new, fully functioning organs through stem cell therapy. Widespread regenerative medicine will need multidisciplinary and international cooperation. Another challenge is immune rejection in stem cell transplantation. Sometimes the immune system misidentifies transplanted cells as foreign organisms, resulting in transplant or cell rejection. One idea for making stem cells "safe" is for them to self-destruct if they become dangerous. Despite these daunting obstacles, stem cell research is making tremendous strides every day. Untreatable neurodegenerative disorders may be able to be treated using stem cell treatment in the near future. This technique allows for the utilization of the patient's own stem cells. In the fight against current and future illnesses, tissue banks are becoming more popular since they collect cells that are the source of regenerative therapy. Human life may be extended more than ever before thanks to stem cell treatment and its rejuvenation advantages [12, 77, 87].

11. MISCONCEPTIONS BETWEEN PRP AND STEM CELLS

Although intriguing, regenerative medicine remains a misunderstood area. In this regard, it's not surprising that many individuals mistakenly believe stem cell therapy and PRP treatment are the same. Because of the similarities in function and the fast preparation of PRP,

Table 2. Characteristic features of stem-cell treatment and platelet-rich plasma [108-110].

Feature	Stem-cell Therapy	Platelet-rich Plasma
Source for treatment	Adult stem cells such as bone marrow, adipose tissue, umbilical cord tissue, umbilical cord blood, and placental tissue. Embryonic stem cells could be controversial. The source could be varied.	Venous blood from own blood.
Key difference	Regenerate tissue that is lost and accelerate the body's healing process by secreting paracrine factors.	Accelerate the body's existing healing process.
Components of injection	Undifferentiated cells.	Platelets, white blood cells, growth factors, catabolic cytokine (VEGF, EGF, TGF-β1, PDGF-AB, MMP-9, IL-1β, etc.)
Mechanism of action	This stem cell solution is injected into the problem area and triggers a healing response. Stem cells can even form new tissue, regenerate damaged or diseased tissues, reduce inflammation and modulate the immune system.	Platelet activation for the healing process and anti-inflammation
Site of administration	IV Stem Cell Therapy (Intravenous administration), Intrathecal (directly into the spinal canal), site injections into problem areas	Injection at injured or diseased body tissue.
Potential risks and suggestions	Allogenic stem cells and cells contaminated with xenogeneic components could cause transplant rejection and hyper-immunogenicity. Hence, the use of xenogeneic components during *in vitro* expansion or processing of stem cells should be avoided.	Bovine thrombin produces antibodies against human clotting factors V, XI, and thrombin, raising the risk of catastrophic coagulopathies. Before starting therapy, check for coagulopathies and platelet dysfunction. It would be better to avoid the use of bovine thrombin in PRP therapy if possible.

many doctors convince patients to choose PRP-based methods, claiming that it is comparable to stem cell therapies. While these two therapies are often used to treat a wide range of orthopedic ailments and injuries, these treatments are commonly employed in both cases. In both treating injuries and dealing with pain, these two techniques have many similarities, but they are not the same as depicted in Table **2**. Furthermore, the effectiveness and persistence of preparative techniques, agreement in preparative methods across various research groups for clinical applications, and the relevance of such technologies as a replacement for traditional treatments should be defined and properly applied.

12. MONITORING OF REGENERATIVE MEDICINE BY REGULATORY BODIES

Regenerative therapy is a rapidly evolving medical field that uses stem cells, gene therapy, and other biologic materials to regenerate or replace damaged tissues and organs. As a result, regulatory bodies such as the Food and Drug Administration (FDA) of the United States, the European Medicines Agency (EMA) of the European Union, the Pharmaceuticals and Medical Devices Agency (PMDA) of Japan, the Therapeutic Goods Administration (TGA) of Australia, and the Health Canada have developed guidelines to ensure the safety and efficacy of regenerative therapies [111-115]. These guidelines require that regenerative therapies be subject to rigorous preclinical and clinical testing before they can be approved for use in humans. This includes testing the therapies for safety, efficacy, and quality, as well as monitoring the long-term outcomes of patients who have received these therapies.

Besides, preparing a guideline, the regulatory bodies also require to monitor that regenerative therapy products are being manufactured under strict quality control standards to ensure their safety and efficacy. This includes ensuring that the stem cells used in the therapies are of high quality and free from any kind of contamination or genetic alteration. Furthermore, following approval of a regenerative medicine product, regulatory bodies require to monitor continuously the safety and efficacy of the approved product(s) through post-market surveillance. This may involve tracking adverse events reported by patients or healthcare providers, as well as conducting additional clinical trials to further evaluate the therapy's long-term safety and efficacy. The local regulatory body should educate the people regarding regenerative medicine products and should aware them not to take any product that has not received regulatory approval or not to take part to any clinical trials that have not received ethical approval from the local ethics approval committee.

CONCLUSION

Platelet-rich plasma and stem cell-based therapy are becoming very popular in the field of regenerative therapy. Because of easy access and can be prepared from the patients' blood, PRP became a very popular tool of regenerative medicine. Platelet-rich plasma can promote regeneration by providing growth factors or other paracrine factors. However, where a large number of cells are required to replenish the need to regenerate tissues and/or organs, cell-based therapies are the preferred option. Furthermore, other tools or techniques of regenerative medicine, such as bioprinting, organoids, and mechanobiology, also require stem cells for success. Hence, the people involved in regenerative medicine should not misguide people by saying PRP as a cell-based therapy or should not show it as complementary to stem cells.

The speed of research continues to accelerate, requiring continuous investment from fundamental to clinical science to offer resources for innovation. While there are many possibilities in many areas, such as neurological or metabolic diseases, difficulties are increasing. One issue persists: commercial clinics provide unregulated goods and services that claim a broad variety of advantages but utilize poorly defined therapies with little proof of efficacy, possible safety issues, a hazy scientific justification, and the main goal of financial profit. Thus, proper monitoring by the regulatory bodies should be the highest priority to maintain the quality of the products and services provided using the tools of regenerative medicine.

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