Dermatological Reviews

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Dermatological Reviews

INVITED REVIEW

Basic Science and Principles of Stem Cells, Platelet-Rich Plasma, and Exosomes

Elliott Lyles[1] | Carol Stegura[2] | Luke Broughton[2] | Alan Snyder[3] | Todd Schlesinger[4,5]

[1]College of Graduate Studies, Medical University of South Carolina, Charleston, South Carolina, USA | [2]College of Medicine, Medical University of South Carolina, Charleston, South Carolina, USA | [3]Department of Dermatology and Dermatologic Surgery, Medical University of South Carolina, Charleston, South Carolina, USA | [4]George Washington University School of Medicine and Health Sciences, Washington, DC, USA | [5]Clinical Research Center of the Carolinas, Charleston, South Carolina, USA

Correspondence: Todd Schlesinger (skindoc@dermandlaser.com)

Received: 25 July 2024 | **Accepted:** 30 July 2024

Keywords: exosomes | extracellular vesicles | platelet-rich plasma | regenerative medicine | stem cell therapy

ABSTRACT

Background: Regenerative medicine seeks to harness the natural healing abilities of the body and has scope for use in the treatment of dermatologic conditions. Stem cell therapy, platelet-rich plasma (PRP), and exosomes are emerging as key components in regenerative medicine, particularly in skin rejuvenation and repair.

Objectives: The goal of this article is to review the basic science behind the function of stem cells, PRP, and exosomes in regenerative medicine.

Methods: A literature review was conducted using PubMed and Google Scholar, focusing on basic science literature regarding the structure, origin, mechanisms of action, and isolation of stem cells, PRP, and exosomes.

Results: The evaluation found that stem cells facilitate skin regeneration by modulating the inflammatory milieu. PRP was shown to have positive effects on repairing skin elasticity and improving scars through the release of growth factors. Exosomes were found to enhance fibroblast proliferation and collagen synthesis.

Conclusions: Stem cell therapy, PRP, and exosomes each show mechanisms that render them useful in regenerative medicine. Future research is needed to further elucidate their mechanisms of action, standardize their isolation procedures, and optimize their potential for clinical application.

1 | Introduction

Regeneration of living tissues and organs is a combination of stem cell biology, tissue engineering, and cell transplantation [1]. In recent decades, these fields of study have intertwined and ultimately produced biological replacements capable of repairing manifestations of disease. Some of these biological replacement tools include stem cells, platelet-rich plasma (PRP), and exosomes.

Stem cells are famously studied for their pluripotent properties, allowing for differentiation into multiple cell lineages, and capability to repair the tissue of choice through regeneration. PRP can secrete trophic factors into the tissue to support and stimulate the process of regeneration. Exosomes are intercellular communicating vesicles that carry cargo, including proteins, nucleic acids, and lipids. Transportation of target cargo can be an effective mechanism to stimulate tissue regeneration. Although stem cells, PRP, and exosome therapy have similar end goals, there exists a difference in patterns of origin, isolation methods, structure, and mechanisms of action.

Stem cells, PRP, and exosomes have all been applied in the field of dermatological regenerative medicine. DNA damage in the

Elliott Lyles and Carol Stegura contributed equally to this work and should be listed as co-first authors.

skin can be due to ultraviolet radiation, reactive oxygen species, and other forms of trauma [2]. Aging of the skin leads to loss of repair mechanisms and therefore, use of stem cells, PRP, and exosome has become an attractive option. Dermatological applications include skin rejuvenation and wound healing. All three modalities share mechanisms to increase angiogenesis in the dermis, increase collagen production, and increase fibroblast production. There are also similarities in their outcomes when applied with other therapies such as microneedling and lasers.

2 | Stem Cells

Stem cell therapies have been used for many medical applications, such as bone marrow transplants for different blood and immune disorders, and more recently, have increasingly been used in regenerative medicine. A stem cell can both self-renew and regenerate differentiated cells in a variety of tissues from an embryo, fetus, or adult, and can be intentionally produced from genetically programmed cells, termed induced pluripotent stem cells [3]. Stem cell potency refers to the differentiation capabilities of the cell. Totipotency is the propensity for a stem cell to differentiate into any tissue type. Pluripotency is the ability to recreate multiple cell lineages and any tissue in the body excluding the placenta. Multipotency describes the capability to create multiple types of cells in a specific lineage; meanwhile, bipotency describes the capacity to create two different types of cells and unipotency refers to creation of one type of cell [4].

2.1 | Origins

Stem cells derived from embryonic tissue can be harvested from the morula delivering totipotent capabilities or from the subsequent blastocyst with pluripotent properties. Totipotent cells evolved from the morula differentiate into its first two lineages: the inner cell mass and trophectoderm. Pluripotent cells are found in the blastocyst's inner cell mass and, as the zygote matures, cellular totipotency continues to diminish. Nonetheless, these pluripotent embryonic cells can still differentiate into all three germ layers: the endoderm, mesoderm, and ectoderm [5].

Stem cells derived from the fetus, amniotic fluid, placenta, and Wharton's jelly possess a combination of multipotent and pluripotent properties. Fetal stem cells can be isolated from fetal blood, bone marrow, liver, and kidney. Adult stem cells are present in fully developed tissues and can be derived from fat, bone marrow, skeletal muscle, skin, and blood. Hematopoietic stem cells (HSCs) were previously identified in 1961 as the adult stem cell derived from blood that proved successful in bone marrow transplants [6]. Another type of adult stem cell is a mesenchymal stem cell (MSC) sourced from bone marrow, adipose tissue, or umbilical cord tissue and can differentiate into mesenchymal tissue lines. MSCs are considered a multipotent cell that uniquely possesses pluripotent properties, whereas most other adult stem cells only possess multipotent, bipotent, or unipotent properties [4]. Pluripotent properties allow for less restriction of lineage differentiation potential compared to multipotent cells. MSCs' pluripotent properties are of interest to researchers because they do not possess associated risks of immune rejection and teratoma formation that pure pluripotent cells do [7]. Although MSCs can be isolated from many different types of tissues, Wharton's jelly and other early life tissues are thought to be more proliferative, immunosuppressive, and therapeutically active due to their limited age of development [8].

2.2 | Methods of Stem Cell Isolation

Embryonic stem cells have previously been isolated from inner cell mass using immuno-surgical techniques consisting of the incubation of a blastocyst with trophectoderm antibodies. This is then introduced into complement proteins, resulting in lysis of the trophectoderm, so the only surviving cells are in the inner cell mass [9]. Somatic cell nuclear transfer is a process where the oocyte nucleus is removed in culture and replaced with a somatic cell nucleus from a patient. The cell taken from the patient and combined with a donated oocyte incorporates the patient's DNA and allows for patient specificity. The cell is then divided to the blastocyst stage by chemical and electrical signals. The inner cell mass can then be isolated, resulting in embryonic stem cells identical to those of the patient [10]. Single-cell embryo biopsy is another method that is patient-specific and does not create or destroy embryos. Arrested embryos can also be used, but a limitation of verification of the appropriate quality of the cell line remains. Due to the possibility of cloning using this technique, there are ethical concerns. These concerns have led to the development of “altered nuclear transfer,” which is a variant of somatic cell nuclear transfer that restricts clonality. This method genetically modifies a nucleus from a somatic cell, which is then transferred into a human oocyte so that a genetic defect is introduced such that the cell is incapable of forming a clonal zygote [11].

In regenerative medicine, alternative approaches to adult MSC isolation are used, such as reprogramming. Reprogramming dedifferentiates adult somatic cells to create pluripotent stem cells specific to patients without the use of embryos [12]. There is a risk of cancer formation when mutations of oncogenes and tumor suppressor genes are induced during the conversion of stem cells into cancer cells [13]. Fibroblasts have previously been activated for Oct-4 and Nanog genes, allowing them to show similar properties to embryonic cells [14]. Oct-4 is one transcription factor that supports self-renewal and pluripotent properties. Oct-4 exists in the inner cell mass as well as embryonic stem cells, embryonic germ cells, and embryonic carcinoma cells. Oct-4 deficiency in mice has shown lack of development past the blastocyst because no inner cell mass cells have been able to develop [15].

The adult stem cells that have been studied the most are the $CD34^+$ hematopoietic stem cells isolated from the bone marrow. Hematopoietic stem cells can regenerate all hematopoietic cell lines and are widely used in transplants for patients with defective or depleted bone marrow. Hematopoietic stem cells can be isolated through selection of cells expressing $CD34^+$. These cells can be targeted using an antibody-dependent or non-antibody-dependent method. Common techniques include centrifuging a cell suspension to remove red blood cells [16].

MSCs can be isolated using similar centrifugation techniques, but have diverse markers from hematopoietic stem cells. MSCs demonstrate (CD73, CD105, and CD90) and lack less specific surface markers (CD45, CD34, CD14 or CD11b or CD79α or CD19, and HLA-DR) [17]. Isolation is dependent on their ability to adhere to plastic surfaces. Bone marrow-derived MSCs can be washed, counted, and suspended in culture medium. After 24–72 h (about 3 days), the medium is changed, which removes nonadherent cells. After 1 week, bone marrow stroma is formed and is maintained by biweekly medium changes. This results in the elimination of all nonadherent cells and a sample of MSCs that can be used in hematopoietic stem cell transfer [18].

Adipocyte-derived stem cells (ASCs) can be isolated from adipose tissue that has either been removed surgically through liposuction or resection [19]. Currently, no standardized protocol is in place. The most widely used method described by Zuk et al. consists of washing samples in phosphate-buffered saline and denaturation with 0.075% collagenase at 37°C for 30 min [20]. After this step, multiple cell types will exist in a medium called stromal vascular fraction (SVF). Dulbecco's modified Eagle's medium is then added to neutralize the SVF. For lysis of red blood cells, the sample is centrifuged and then suspended in NH_4 Cl and incubated at room temperature for 10 min. Lastly, the SVF is filtered through a nylon mesh and incubated at 37°C overnight and 5% CO_2. Until adherent cells reach sub-confluence, they are kept under standard conditions. At this point, harvestable SVF can be utilized in vitro and differentiated into adipogenic, chondrogenic, and osteogenic cells.

2.3 | MSCs' Mechanism of Action

MSCs are immunomodulatory cells that operate through cell contact-dependent mechanisms and soluble factors [21]. MSCs interact with monocytes and regulatory T cells to promote the determination of monocytes/macrophages toward an anti-inflammatory response. MSCs can produce interleukin (IL)-6 and hepatocyte growth factor, both of which increase the inflammatory monocyte's release of IL-10, thereby preventing monocyte differentiation into dendritic cells and adding to the anti-inflammatory response [22]. These monocytes also express MHC class II, CD45R, and CD11b, which suppress T-cell activity. MSCs have also been shown to inhibit the migration and maturation of dendritic cells. Interestingly, dendritic cells cannot support $CD4^+$ T cell proliferation in the presence of MSCs, compared to when they are not in the presence of MSCs. After the intravenous (IV) administration of MSCs, they become caught in the capillary lung system. The MSCs are then phagocytosed by macrophages and generate the same anti-inflammatory response [23]. MSCs also suppress $CD4^+$ and $CD8^+$ T cells. MSCs activate Th2 cells, which produce anti-inflammatory cytokines and increase concentrations of regulatory T cells downregulating mechanisms of autoimmunity. MSCs can also upregulate the formation of regulatory B cells and downregulate plasmablast formation, which may play a role in establishing immune tolerance and produce additional IL-10 [24]. MSCs can additionally inhibit natural killer cells from proliferating [23]. All these mechanisms synergistically drive an anti-inflammatory response and can be used for a many different types of regenerative and anti-inflammatory therapies.

2.4 | Future Applications and Challenges

Embryonic stem cells have shown benefits for diabetes, heart disease, cerebrovascular disease, liver and renal failure, spinal cord injuries, and Parkinson's disease [10]. However, embryonic stem cells are avoided due to the risk of formation of teratomas due to uncontrolled proliferation, immunological response if not patient-specific, and ethical concerns. These ethical issues include controversies over what is considered the onset of life and if human reproduction must be necessary. While researching on embryonic stem cells, destruction of embryos and creation for research purposes are also debated upon. Reprogramming cells to produce pluripotent effects avoids this argument, but other issues remain. Donation of biological materials and stem cell clinical trials must exist with informed and voluntary consent [25].

Adult stem cells, hematopoietic stem cells, and MSCs are the most widely used in regenerative medicine due to ease of isolation from diseased individuals and the absence of ethical concerns [26]. However, there are still challenges, including the publicization that adult stem cells are exceptional sources of plasticity, transdetermination, and transdifferentiation, when many trials have shown only marginal benefits. Some evidence points to the effects being more paracrine than regenerative [7]. Fully understanding the mechanism of stem cells is extremely important. Efficiency must also be refined, and the scale of procedures must be simplified [27].

Future applications include using the reprogramming method to delete certain genes from the stem cell to decrease the risk of development of teratomas. Induced pluripotent cell technology has also expanded the future for regenerative medicine due to its ability to reproduce all three germ layers without destroying embryos. Other future research could consider how to best generate new tissues by fine-tuning tissue engineering mechanical and physiochemical properties of scaffolds [4].

2.5 | PRP

PRP is an autologous concentration of platelets in a small volume of plasma. It is derived from the patient's own blood and is characterized by a platelet count significantly higher than baseline levels found in circulating blood. These levels can vary from 2 to 3× or from 5 to 9× baseline concentrations depending on the device used [28]. However, PRP is currently characterized by its total platelet concentration, with a minimum concentration of $1 \times 10^6/\mu L$ [29]. After discovering tissue response to injury and the release of important bioactive molecules for healing in the 1980s, PRP began to gain popularity for its use in tissue repair and regenerative medicine [30]. Platelets contain a variety of active growth factors and proteins for processes such as the hemostatic cascade, synthesis of new connective tissue, and revascularization. The idea behind the therapeutic approach of PRP lies in the injection of a much higher amount of platelets, which facilitates and accelerates the healing process. Historically, clinical preparation of PRP has not been standardized, with techniques varying by clinician [29]. To further complicate the ability to standardize this procedure,

different types of PRP are used depending on the clinician. The first is pure platelet-rich plasma (P-PRP), which is a leukocyte-poor preparation with a low-density fibrin network [28]. Leukocyte-rich PRP (LR-PRP) contains the same product as PRP but with an increased number of leukocytes. It is worth noting that this preparation is the most common among the commercial kits available. Pure platelet-rich fibrin (P-PRF) does not contain leukocytes but contains a high-density fibrin network. This preparation is only available as a gel and therefore cannot be injected like P-PRP and LR-PRP. Leukocyte-rich PRF (LR-PRF) contains both leukocytes and a high-density fibrin network. Additionally, PRP content differs from person to person, further complicating the ability to create a standardized procedure. This has led to discrepancies in the number of platelets collected from plasma and possibly the efficacy of treatment. PRP has been used as a therapeutic modality in multiple different specialties, including cardiac surgery, pediatric surgery, gynecology, urology, plastic surgery, and ophthalmology [31]. PRP also functions as a novel therapeutic modality in dermatology and is being used in patients for various purposes, such as skin rejuvenation, hair growth, and acceleration of wound healing [32]. PRP is administered directly into the skin or scalp, where it can exert its regenerative effects. These treatments will be explored and further expounded on in this review.

The therapeutic efficacy of PRP is primarily due to the high concentration of growth factors and cytokines present in platelets. These growth factors are present in the granules within platelets, namely, the alpha granules and dense granules, which are released upon activation [33]. The alpha granules contain many of these growth factors, including platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF), and epidermal growth factor (EGF), which contribute to the various regenerative mechanisms through stimulating processes such as angiogenesis, cell proliferation and differentiation, and chemotaxis [29]. The dense granules contain molecules such as serotonin, histamine, dopamine, calcium, and adenosine, which may increase the permeability of cell membranes and decrease inflammation [32]. Upon administration, PRP contains these bioactive molecules, which are responsible for the beneficial response seen in treatment. PRP enhances the proliferation of fibroblasts and the release of proteins involved in tissue repair and regeneration, leading to increased cellular turnover and repair. Additionally, the growth factors in PRP stimulate the production of collagen through PDGF, a crucial structural protein in the extracellular matrix of the skin, thereby improving skin texture and elasticity. VEGF in PRP promotes angiogenesis, enhancing the blood supply to treated areas and supporting tissue vitality and regeneration [30]. Furthermore, specifically in PRP for osteoarthritis treatment, PDGF and TGF-β have both been seen to reduce inflammation. The exact mechanisms for inflammation induction and inhibition are elusive, as PRP preparations contain a mixture of pro- and anti-inflammatory molecules. For example, Lin et al. reported that PRP treatment reduces local inflammation by inhibiting chemokine transactivation and CXCR4-receptor expression [34]. In short, PRP can modulate the inflammatory response and induce a robust healing process.

2.6 | Preparation Protocol

Multiple techniques are used to collect PRP, and reproducible results have been seen using differential centrifugation [35]. The two main methods of separating PRP from whole blood are the PRP method and the buffy coat method, both of which utilize the process of differential centrifugation. The PRP method utilizes blood from venipuncture, which is then spun at a lower speed to separate the red blood cells. This is followed by a spin at higher speeds to separate the platelet concentrate. The other method is the buffy coat technique, which involves storing whole blood at room temperature and then spinning at high speeds to yield three separate layers: RBCs, platelets and white blood cells, and platelet-poor plasma. The buffy coat is separated and then spun at a low speed to separate the white blood cells and obtain the PRP. These protocols produce similarly efficacious samples of PRP, and the most important factor in obtaining the necessary platelet yield is the centrifugation rate. The platelet-rich layer (buffy coat) contains a significantly elevated concentration of platelets compared to whole blood. In certain protocols, PRP is activated using agents such as calcium chloride and thrombin to induce the release of growth factors from the platelets before injection [36]. The term “activation” mainly refers to two processes: calcium chloride causes the degranulation of the platelets to release the growth factors mentioned above and thrombin allows for the cleavage of fibrinogen, which leads to matrix formation, confining the treatment to a localized area [37]. Alternatively, activation may occur in situ following administration into the tissue.

PRP therapy is a unique therapy in the sense that it uses the patient's own biological resources to promote healing and tissue regeneration, offering a minimally invasive and autologous treatment option with a minimal risk of adverse reactions. Because of this, it has been proven to be beneficial in dermatologic procedures such as androgenetic alopecia, scar revision, skin rejuvenation, and dermal augmentation [31].

2.7 | Challenges of PRP and the Future of PRP Therapy

PRP has been used across multiple fields in medicine for the treatment of various diseases and has been proven to be useful in the treatment of various conditions such as AGA, scar treatment, osteoarthritis, and more. However, the lack of standardization of PRP collection has made comparison of data between different studies difficult, and more studies using a standardized procedure need to be carried out [35]. Furthermore, as PRP samples are autologous, results seen in individuals may vary greatly, as seen in studies using PRP treatment for AGA [31]. Additionally, genetic polymorphisms may further determine whether or not a patient will respond to PRP treatment. Future studies should aim to elucidate how to maximize the effects of PRP, such as using $CD34^+$ enriched PRP or other preparations by conducting RCTs. However, the autologous nature of PRP treatment may be both a benefit and a hindrance. Although it is highly unlikely for patients to have an adverse reaction to PRP as it is an autologous form of treatment, the number of platelets, GFs, and other necessary elements for significant results may vary from person to person. More

research in controlled trials may help to pave the way for standardization of PRP collection and therefore more consistent results among different providers.

3 | Exosomes

Extracellular vesicles (EVs) encompass three subtypes of nanoparticulate vesicles: exosomes, microvesicles (MVs), and apoptotic bodies. EVs can be further categorized on the basis of their intracellular origin and size. Exosomes are typically 30–150 nm in size, endocytic in origin, and are critical for intercellular communication in both normal physiology and pathologic conditions. Exosomes, which are released by all cells and are found in bodily fluids, have gained significant attention in research for their implications in disease diagnostics and treatment [38, 39].

It is well known that cells communicate through hormones, neurons, paracrine signaling, and direct contact to maintain homeostasis and respond to stressors in their environment. Such communication also occurs as cells release EVs, which are inherently specific to the needs of the parent cell. Exosomes contain information in the form of nucleic acids, proteins, lipids, and metabolites that function to affect cellular processes [40].

Exosomes have been reported to play a role in regenerative medicine, such as in terms of their ability to decrease inflammation, reverse fibroblast senescence, and promote angiogenesis [41, 42]. Structurally, exosomes are flexible and small, making them amenable for topical use, and can be used as monotherapy or serve as an adjunct therapy [43, 44]. An increasingly common dermatologic procedure, microneedling-drug delivery, can be used concomitantly with topical exosome application to increase tissue permeation and increase efficacy [45].

3.1 | Exosome History

"MVs" were first described by Peter Wolf in 1967 as a byproduct of platelet ultracentrifugation [46]. In 1971, Leville Crawford found that platelet-derived "microparticles" contained lipids and proteins [47]. It was not until 1981 that the term "exosome" was used to describe vesicles of 40–1000 nm [48]. In 1983, exosomes were specified to be smaller, about 100 nm, in size and released from reticulocytes through a multivesicular body (MVB) as a mechanism to selectively eliminate receptors during differentiation [49, 50]. The understanding that exosomes functioned to remove waste developed to include their role in antigen presentation, as exosomes were also found to originate from lymphocytes and dendritic cells [51, 52]. Exosomes were soon found to originate from mast cells, neutrophils, epithelial cells, and tumors [53–56]. In addition to proteins and lipids, exosomes were capable of carrying RNA to target cells that resulted in phenotypic changes [57, 58]. Specifically, glioblastoma-derived exosomes were reported to transport mRNA and angiogenic peptides that contributed to the proliferation of other glioblastoma cells. Additionally, glioblastoma-derived exosome contents were reported in the serum [58]. This discovery highlighted the role of exosomes in intercellular communication and underscored their potential in cancer diagnostics.

The overlap in EV composition makes separation difficult and the challenge is compounded by the lack of standardized isolation techniques. This challenge contributed to years of confounding literature and misidentification of EVs [38, 59, 60]. Although there has been a substantial effort over the last decade to standardize EV terminology, it is important to interpret exosome research findings with caution, as findings may be better generalizable to all EVs [39, 60–62].

3.2 | Exosome Structure

Exosomes are phospholipid, bilayered spheres that are secreted into the extracellular space. Exosomes are 30–150 nm in size, 1.13–1.19 g/mL in density, and are made of cholesterol, phospholipids, and sphingomyelin [63]. Exosome membranes contain higher lipid concentrations than their parent cell membranes; the individual lipid concentrations are similar among exosomes regardless of their parent cell origin [64, 65].

Lipids are quintessential exosome components, as they contribute to its structure, membrane integrity, biogenesis, and homeostasis [64, 66]. For instance, exosomes have similarities to detergent-refractory membranes that allow them to survive in the extracellular space [67]. The exosome membrane protects its internal cargo from degradation, such as protecting RNA from circulating ribonucleases, allowing them to have distal effects [68]. Additionally, lipids in the exosome membrane induce bud formation from the MVB to form intraluminal vesicles (ILVs) during their biogenesis [69].

Exosomes contain several surface molecules, including tetraspanins, immunoglobulins, receptors, and ligands [70]. Tetraspanins are transmembrane proteins with two extracellular loops and two intracellular tails; the most commonly identified exosomal tetraspanins are CD9, CD63, and CD81 [71]. These tetraspanins are also found in other types of EVs and are not specific to exosomes [72]. Other membrane proteins include membrane transport proteins, adhesion molecules, integrins, major histocompatibility complexes, fusion proteins, and proteins for biogenesis [39, 66, 73, 74]. Due to these membrane proteins, exosomes have the unique ability to target specific cells as well as facilitate exosome cargo uptake [75].

3.3 | Exosome Biogenesis

Other EVs, such as MVs and apoptotic bodies, form through an exocytic pathway and bud from the parent cell membrane, whereas exosomes form from an endocytic pathway [76]. In 1983, Harding et al. first described a receptor-mediated endocytic pathway that is now well recognized as the exosome secretion pathway. Eventually, the MVB fuses with the parent cell membrane, releasing the ILVs into the extracellular space, where they are referred to as exosomes [77, 78].

Exosome formation begins as proteins and lipids along the parent cell membrane and immediate extracellular space bud inward from the plasma membrane to form an intracellular early endosome. With interactions between the golgi apparatus and the endoplasmic reticulum, the early endosome matures into a late endosome [79, 80]. During this maturation process, the endosome membrane exchanges sphingomyelin for ceramide and Rab5 is substituted for Rab11 [81, 82]. Additionally, the endosome plasma membrane begins to invaginate to create ILVs that contain cargo; as such, ILVs are double-invaginations from the plasma membrane of the parent cell [39]. ILVs are analogous to "early exosomes," and an endosome with ILVs is referred to as an MVB [83].

The endosome pathway leads to fusion with a lysosome for degradation or fusion with the plasma membrane for expulsion. It is thought that the lipid concentration influences which path the endosome is directed toward. Early in endosomal maturation, ILVs have a higher concentration of cholesterol compared to more mature ILVs. Exosomes have a high concentration of cholesterol in their membrane; thus, it is thought that exosomes form during early maturation [84]. Interestingly, MVBs that have lower internal cholesterol levels are directed to the alternate endosomal pathway: lysosomal destruction [85].

Although complex, there are two better defined pathways by which an ILV is loaded with its specific cargo: the cytoplasmic endosomal sorting complex required for the transport (ESCRT)-dependent pathway and the ESCRT-independent pathway [86, 87]. The ESCRT-dependent pathway consists of class E vacuolar protein sorting (Vps) proteins that combine to make ESCRT complexes 0 through III. Each of the four complexes works in a consecutive fashion to assemble, bud, and dissociate the exosome from the parent cell. Moreover, Vps4 plays key roles in ILV formation during maturation and complex dissociation during exosome expulsion [70].

ESCRT-associated proteins include ALIX, TSG101, HSC70, and HSP90B. Interestingly, because ESCRT proteins and their associated proteins are involved in exosome generation, these proteins are found in exosomes regardless of the parent cell. Thus, these protein markers are used as exosome biomarkers [38, 88].

Proteins and lipids allow ILV formation in an ESCRT-independent pathway. Tetraspanins are involved in numerous steps as well as ceramides and sphingolipids [87, 89]. For example, lipid species accumulate to form lipids rafts, which induce curvature in the endosome membrane, facilitating ILV formation [87]. Several Rab-associated GTPases function to coordinate MVB trafficking and fusion with the parent cell plasma membrane [90]. Specifically, Rab7, Rab11, Rab27, and Rab35 have been implicated [88, 91–93]. Studies have shown that the soluble NSF-attachment protein receptor (SNARE) complex and its associated proteins coordinate MVB fusion with the plasma membrane [94]. These mechanisms are still unclear in the scientific literature and likely vary among different cell types, indicating their cell-type specificity [95, 96]. This specificity is seen in melanoma-derived exosomes, as their biogenesis is unaffected by loss of ceramide synthesis, emphasizing that ESCRT-independent pathways are cell-type specific [97].

ESCRT is a ubiquitin-dependent process and is associated with ubiquitin proteins such as Hrs, STAM1, and TSG101 [98]. Proteins, nuclear content, and lipids are each identified and loaded as cargo differently. For instance, some proteins must be ubiquitinated to enter the endosomal pathway [99]. RNA has several different proteins, termed RNA-binding proteins, that give RNA the appropriate signal to then be loaded into exosomes [100].

3.4 | Exosome's Mechanism of Action

There are different mechanisms by which an exosome docks and communicates with its target cell membrane. The exosome can communicate via a target cell membrane receptor that elicits an intracellular response, it can fuse with the target cell membrane, delivering its cargo directly inside, or the exosome can be internalized through endocytosis [101–103]. There are scenarios where exosomes interact with their target cell indirectly via a soluble exosomal ligand. For example, cancer cells have been found to release CD46, a complement inhibitor, via exosomes to confer complement resistance among neoplastic cells [101].

Exosomal cargo communicates with the target cell by either directly stimulating the cell membrane ligands, by transferring activated receptors, or by delivering proteins, lipids, or RNA that reprogram the target cell [104]. The various methods that exosomes use to interact with target cells vary based on the exosome's parent cell. Exosome-mediated immune regulation is commonly communicated through receptor–ligand interactions; for example, dendritic cell-derived exosomes bind toll-like receptors on bacteria to increase the inflammatory response and bind TNF receptors to reduce cell apoptosis [105, 106]. One mechanism by which exosomes directly deliver their cargo is through fusion with the target cell and is speculated to be a route for exosomes targeting tumor cells, as seen in melanoma cells [107]. A recently identified mechanism of interaction, termed filopodia, was reported by Heusermann et al. in 2016. Filopodia are actin filaments protruding from the target cell surface that guide exosomes toward the membrane, where they are then endocytosed [81]. This mechanism has been reported in fibroblasts and resembles bacterial and viral entry [108]. One of the most common ways by which exosomes interact with target cells is through direct internalization of the exosome into the cell. This process is fast and increased by high temperatures [109, 110]. Exosomes that interact via direct internalization are commonly routed for lysosomal degradation, and thus their cargo is released into the cytoplasm of the target cell. If not degraded by a lysosome, exosomes can remain in the endocytic pathway by entering the cell and joining with an early endosome. In this way, exosome uptake and secretion are often intermingled, and the exosomes being released from cells are a combination of new and recycled exosomes [39, 111]. Although still unclear, the exosome and target cell interaction is not random. Tropism is likely due to parent cell type as well as specific recognition between exosome and target cell membrane components [110, 112, 113].

Endocytosis, or direct internalization, of exosomes includes mechanisms characterized by clathrin, lipid rafts, caveolin, pinocytosis, and phagocytosis. Importantly, all endocytosed

exosomes enter the cell via an endosomal vesicle made of the receiving cell's plasma membrane. Therefore, the vesicle must leave the endosome pathway to allow the exosome to exert its intended effect; this process is known as “endosomal escape” [112, 114, 115]. Several mechanisms for endosomal escape have been studied, such as the physiologic endosomal acidification that activates exosome cargo, passive diffusion of cargo through the cytoplasm, trafficking of exosomes to the endoplasmic reticulum, and fusing exosome-containing endosomes with late endosomes via endosome penetration [103, 116–118].

Clathrin-mediated endocytosis involves the accumulation of target cell membrane proteins and receptors to form clathrin-coated vesicles that internalize exosomes with subsequent fusion into an endosome. Clathrin-mediated endocytosis is seen in many cell types and is the most highly regulated exosomal uptake mechanism [119]. Similarly, calveolin-mediated endocytosis is mediated by the accumulation of calveolin proteins along the target cell membrane that induce membrane invaginations [120]. This mechanism involves proteins, such as dynamin-2, that are found in clathrin-mediated endocytosis [121].

Clathrin and calveolin-mediated endocytosis are limited in their ability to consume clusters of exosomes; this is overcome by pinocytosis and phagocytosis, which are endocytic pathways that form large vacuoles and can engulf groups of exosomes [122]. Macro- and micro-pinocytosis are both dependent on growth factors such as epidermal growth factor receptor stimulation [123, 124]. Macro-pinocytosis is driven by lamellopodia and receptor tyrosine kinase-induced membrane ruffling that form pinosomes and subsequently fuse with the endosome pathway [123, 125]. Lastly, phagocytosis depends on phosphatidylinositol-3-kinase and phospholipase C for the phagosome to close around exosomes and fuse with a lysosome to form a phagolysosome [126]. Phagocytosis is commonly used by macrophages and dendritic cells to engulf exosomes [127].

3.5 | Exosomes in Regenerative Medicine

Regenerative medicine utilizes cells, biologic cues, and scaffolds to produce an intended effect; exosomes are categorized as biologic cues [128]. Due to their intercellular communicative abilities, exosomes play an important role in regenerative medicine, as they stimulate the body's natural ability to heal itself [129]. Specifically, exosomes do this by modulating the epigenetics of target cells through RNA species and protein [129, 130].

Studies show that exosomes play a role in musculoskeletal regeneration. Inflammatory cytokines that cause osteoarthritis are influenced by miRNAs; exosomal transport of miRNAs has been shown to decrease cytokine concentration, resulting in chondrocyte proliferation and decreasing chondrocyte apoptosis [131, 132]. Exosomes have been implicated in healing tendonitis by increasing matrix metalloproteinases (MMPs) and improving osteoporosis by increasing osteoblast proliferation [133, 134]. Exosomes have also been reported in neurologic regeneration. Intranasal administration of exosomes improved motor function in spinal cord injury through increased angiogenesis and axonal regeneration [135].

Many dermatologic applications of exosomal rejuvenation are under investigation. Ultraviolet (UV) radiation produces various cellular and extracellular changes within the skin, such as thickening of the epidermis, disorganization of collagen and elastic fibers, gene alterations, and morphologic changes of cutaneous cells [136]. Increasing cellular and extracellular components, such as collagen and elastin, are critical for skin rejuvenation [137].

Stem cells are commonly used to obtain exosomes and the stem cell's origin often determines the cargo contained within the exosome. Umbilical cord stem cells (UCSCs) produce exosomes that contain a protein found to mediate UV-induced reactive oxygen species through the SIRT1 pathway [138]. ASC-derived exosomes contain miRNA that modulate fibroblast proliferation, protect against UV radiation, and affect collagen synthesis as well as proteins that increase collagen synthesis. These changes work synergistically to heal human dermal fibroblasts (HDFs) with UV damage [139]. Specifically, ASC-derived exosomes contain miR-1246, which leads to a decrease of target cell MMPs and an increase in the TFG-β/SMAD pathway, resulting in the upregulation of pro-collagen and an anti-inflammatory milieu [140]. Bone marrow stem cell-derived exosomes increase collagen through the MAP kinase pathway and decrease UV-induced reactive oxidative stress [141]. Interestingly, adult stem cells reversed to pluripotent stem cells produce exosomes that reduce the expression of an aging marker: SA-B-Gal. These pluripotent stem cell-derived exosomes also reverse HDF senescence [142, 143].

It is noteworthy that platelets are another source of exosomes and confer advantages to stem cell-derived exosomes. Blood donations are typically used for red blood cells contributing to a relative excess in platelet donations, providing a source of platelets to extract exosomes from. Additionally, platelet-derived exosomes avoid the challenges with cell expansion that is often associated with stem cell-derived exosomes [144, 145]. In a human study, topical administration of platelet-derived exosomes reduced facial erythema and melanin pigmentation in 6 weeks [146]. HDFs produce exosomes that impact growth differentiation factor 11 (GDF11); by increasing the expression of GDF11, HDF-derived exosomes protect HDFs from photo-aging [147].

Exosomes also make for a promising drug delivery vesicle [148]. One of the first applications of this use found that exosomes loaded with curcumin can result in a greater anti-inflammatory effect compared to curcumin without a delivery vehicle [149]. Exosomes can also be engineered to effectively carry miRNA to target cells, such as in cancer [39].

3.6 | Exosome Challenges and Future Directions

The biggest challenge facing exosomes is how to isolate a large sample that is pure and intact. Additionally, the technique should be affordable and timely. Commonly used isolation

methods include ultracentrifugation, ultrafiltration, tangential flow filtration (TFF), immunoaffinity, precipitation, size exclusion chromatography (SEC), and microfluidic technology [150, 151]. Size-based techniques include precipitation, SEC, and microfluidic sorting; these techniques often yield contaminated exosome fractions that erroneously include proteins, lipids, and other EVs [152]. Differential ultracentrifugation is the current gold standard; yet, is complicated by contaminants and shear forces that can damage the exosome [153]. SEC and ultracentrifugation are commonly used together to produce pure and structurally intact exosomes [154]. TFF combines membrane filtration with flow filtration and has been demonstrated to be a time-efficient, reproducible, and scalable isolation technique. Moreover, there is evidence that TFF paired with SEC is superior to ultracentrifugation and SEC [150, 155]. Immunoaffinity techniques are most effective for isolating a specific population of exosomes, as they depend on a target protein or membrane component [156]. Microfluidic technique examples include filtration, inertial lift force, viscoelastic, lateral displacement, acoustic waves, and electrophoretics; these are limited by user ability and operating costs [153]. The heterogeneity of exosomes contributes to the difficulty in standardizing isolation techniques and proteomic analyses suggest that protein-purification methods not be used [157]. The ideal exosome separation method will be able to produce a large quantity of pure exosomes from a specific parent cell [158].

Enzyme-linked immunosorbent assays (ELISAs), polymerase chain reactions (PCR), DNA sequencing, and microanalyses can all be used to characterize exosomes once they have been isolated. Each of these depends on the presence of an exosome marker [159]. Nanoparticle tracking analysis (NTA) has become the gold standard for exosome quantification as it does not depend on a marker. NTA has disadvantages in size discrimination and, like other methods, needs standardization to facilitate reproducibility [153, 160].

Along with isolation and characterization methods, exosome storage techniques have not been standardized. At one point, the International Society of Extracellular Vesicles (ISEV) recommended that EV be stored at −80°C; yet, there is evidence that significant changes occur at this temperature [72, 161]. Several studies have found that the EV concentration and purity decrease, whereas the particle size increases in a time-dependent manner when stored at −80°C [162–164]. Similar to challenges with other regenerative therapies, human-derived exosomes may vary depending on donor demographics, leading to inconsistent results and irreproducibility [165].

Countries all over the world are in the process of developing therapeutic EVs, including natural EVs, engineered EVs, and hybridized EVs. More research is needed to understand the effect of exosomes within the body, especially when delivered systemically. Although studies have demonstrated positive safety profiles, it is important to evaluate the distribution of exosomes within the body and the possibility of unforeseen effects, given that nuclear content may be transferred to unintended cells.

In 2012, the first ISEV conference was held and the National Institute of Health (NIH) developed a common fund initiative titled “Clinical Utility of Extracellular RNA for Therapy Development.” In an effort to organize EV research, the ISEV created the Minimal Information for Studies of EVs (MISEV) in 2014, with revisions in 2018 and the newest release in 2023. These recommendations were originally published to create guidelines for those researching exosomes and have evolved to include the latest in exosome production, separation, and differentiation as well as new discoveries in EV research [60, 166, 167].

In the United States, there are currently no FDA-approved exosome products and clinical applications of exosomes are being used off-label. There are commercially available products, commonly combined with moisturizers or serums, although more research is needed to assess possible conformational changes that could alter the efficacy of these products over time [168].

4 | Discussion

The use of exosomes, stem cells, and PRP represents excellent potential for new biotechnology in regenerative medicine. Exosomes, when compared with PRP and stem cells, offer unique advantages. Due to their small size, exosomes can escape phagocytosis in the liver and spleen but are large enough to remain in the vasculature [169]. Their size also allows them to transfer across barriers, such as the blood–brain barrier [170]. Exosomes can be engineered to deliver drugs, they can be stored over long periods of time, and their long-term use has a positive safety profile to date [171, 172]. In comparison to stem cells, exosomes cannot replicate and thus do not confer the risk of malignant transformation. Unlike stem cells, exosomes do not raise the same ethical concerns of clonality and embryo creation/destruction.

In comparison to exosomes, stem cells can be isolated in large quantities, there are decades of clinical trial evidence, and thus there are FDA guidelines governing their use. A downside to stem cells is that they have the potential to differentiate and lead to the risk of malignant transformation. Stem cells are also difficult to store, graft, and transport and also have the potential to elicit a host immune response, have quality issues before administration, and have a short half-life [171, 173].

The efficacy of PRP depends on the donor; specifically, PRP is affected by donor demographics and lifestyle behaviors such as age and smoking status. Like exosomes, isolation techniques for PRP are not standardized [174]. The variations in the collection and preparation of PRP play a role in its unpredictable clinical efficacy [175]. Additionally, PRP preparations are known to have a short half-life [176].

Synergistic methods using stem cells, PRP, and exosomes have been studied. In a study by Yongyi Zhang in 2024, regeneration after peripheral nerve damage was studied. Although MSC therapy has potential for success, poor engraftment, and neurotrophic effects exist. PRP-derived exosomes were isolated and applied to an environment with MSCs. PRP-derived exosomes were found to significantly increase MSC proliferation, viability, and mobility, as well as decrease stress-induced MSC apoptosis.

MSC quality was maintained and senescence was decreased. In vivo, high therapeutic efficacy was recorded due to an increase in axonal regeneration and remyelination. The PRP-derived exosomes in vitro activated the P13K/Akt signaling pathway in MSCs [177].

Stem cells, PRP, and exosomes are also being used together in engineering skin substitutes. In a study by Yunchuan Wang 2023, type I collagen and PRP were combined to form a scaffold. Adipose MSC-derived exosomes were then added to the scaffold to increase skin performance. This combination scaffold functioned to downregulate inflammation and enhance cell proliferation, angiogenesis, and wound healing. Importantly, the exosomes retained their anti-inflammatory properties when incorporated into the collagen and PRP scaffold [178].

5 | Conclusion

Stem cells, PRP, and exosomes have strengths and promising advancements have been achieved in the field of regenerative medicine. Use of stem cells is a fundamental regenerative method that can target, proliferate, and directly replace tissues. There is potential for PRP to be a valuable tool in regenerative medicine with its numerous metabolites that have a role in regenerative medicine. Exosomes have the unique ability to act as a liaison between cells while transporting bioactive molecules, offering a new mechanism for regeneration. Future research should aim to clarify their mechanisms of action as well as standardize their isolation and characterization techniques. Although there is evidence of their efficacy when using synergistically, further research is necessary to evaluate the effectiveness and feasibility of using these three therapies concomitantly. Stem cells, PRP, and exosomes possess numerous qualities that have added to their success in regenerative medicine.

Acknowledgments

Dr. Todd Schlesinger received an educational grant from wiley for this review.

Conflicts of Interest

Dr. Todd Schlesinger serves as a consultant, investigator, speaker, and/or advisor for Abbvie, Almirall, Allergan (An Abbvie company), ASLAN Pharma, Arcutis, Biofrontera, Beirsdorf, Benev, Bristol-Myers Squibb, Castle Biosciences, Galderma, Eli Lilly, ExoCoBio, Incyte, Janssen, LEO, L'Oreal, Novartis, Pfizer, Regeneron, Sanofi, Sun Pharma, Takeda, UCB Pharma, and Verrica.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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