Nanomed J. 12(1): 1-14, Winter 2025

REVIEW PAPER

The role of platelet derived exosomes in regenerative medicine

Seyyede Fatemeh Shams [1, 2], Mohammadreza Javan [3], Faeze Shahriyari [4*]

[1]Department of hematology and blood banking. Faculty of medicine. Mashhad University of medical sciences, Mashhad, Iran
[2]Cancer Molecular Pathology Research Center, Mashhad University of Medical Sciences,Mashhad, Iran
[3]Department of Anatomy, Physiology and Pharmacology, University of Saskatchewan, Saskatoon, Canada
[4]Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Iranian Blood Transfusion Organization (IBTO), Tehran, Iran

ABSTRACT

Exosomes are a group of extracellular vesicles that are produced by various cells and are abundantly found in body fluids such as plasma. Due to their special structure, these small particles are able to carry and contain various compounds and establish cellular communication. Also, much attention has been paid to the ability of exosomes as new treatment option in various fields of regenerative medicine. One of the most abundant exosomes in plasma is platelet derived exosomes, which are rich in compounds found in platelet granules, such as growth factors. Since the use of platelets and platelet-rich plasma has been very effective in regenerative medicine, in recent years the use of platelet exosomes in regenerative medicine has received much attention and investigation. This review briefly examines the role of platelet exosomes in various fields of regenerative medicine, such as hair repair, wound healing, orthopedic injuries, angiogenesis, and drug delivery. The results of this study show that these microparticles have low immunogenicity, low thrombogenicity, and they are very useful and efficient in regenerative medicine.

Keywords: *Exosomes, Growth factors, Platelets, Regenerative medicine*

How to cite this article
Shams SF, Javan MR, Shahriyari F. The role of platelet derived exosomes in regenerative medicine. Nanomed J. 2025; 12(1): 1-14. DOI: 10.22038/nmj.2024.75580.1836

INTRODUCTION

Regenerative medicine means the restoration of cells, tissues, and organs of the human body from injuries with the help of medical devices, small molecules, complex biological molecules such as cell therapy to obtain their normal function. In regenerative medicine, there is less limitation including lack of donors or immune complications, which are routine in tissue transplantation [1, 2]. The term regenerative medicine was first used in 1992 in an article by Lelander Kaiser that examined the technologies affecting the future of hospitals. After that, this new science made significant progress and the scope of its activities became wider.

There are three different approaches in regenerative medicine: treatment based on cells, use of biological or synthetic materials that cause repair processes and cell growth implanting cellular scaffolds [3]. One of the cells that are widely used in regenerative medicine are platelets and their derivatives such as platelet exosomes. Platelets are non-nucleated blood cells with a diameter of 2-4 µm. They are produced from bone marrow megakaryocytes during the thrombopoiesis process. Rather than hemostasis and thrombosis, they also participate in other physiological and pathophysiological processes.

Activated platelets are able to secrete compounds stored in their granules. Platelets have alpha granules, dense granules and lysosomal granules. These granules contain coagulation and non-coagulant compounds and proteins. For example, alpha granules are rich with platelet factors, endothelial growth factor (EGF), platelet-derived growth factor (PDGF], transforming growth factor-β (TGF-β), and vascular endothelial growth factor/vascular permeability factor (VEGF/VPF). Platelets also make other bioactive compounds such as extracellular vesicles [4,

* *Corresponding author: Email: faezesh550@gmail.com*
Note. This manuscript was submitted on October 12, 2023; approved on January 6, 2024

5]. Therefore, the presence of these compounds in the platelet granules has caused these small blood cells to be of great interest in regenerative medicine. Studies have proven the usefulness of platelets and platelet-rich plasma in various fields of regenerative medicine [6-8]. In the following study, we intend to examine and review the usefulness of platelet exosomes in regenerative medicine. Therefore, we will first define the exosome and the characteristics of platelet exosomes, and then review the effect of platelet exosomes in regenerative medicine.

Platelet- based exosomes

Exosomes are a group of extracellular vesicles that are released from the membrane of living cell, such as platelets, lymphocytes, stem cells, etc.. They are 30-100 nanometers in diameter. At first, these small vesicles were considered cellular debris, but in 1987, they were called exosomes. Since exosomes are actively released by integrating into the cell membrane, they contain various types of compounds such as proteins, mRNA, and microRNA, and play a role in intercellular communication [9]. Exosomes are found naturally in body fluids such as blood, saliva, milk, urine, etc.; so that, they can be effective in both paracrine and endocrine ways [10]. Based on this, it can be said that exosomes are involved in biological activities such as homeostasis, coagulation, inflammation, angiogenesis, removal of unnecessary proteins and mRNA, and pathological activities such as cancers and infections [11-13].

Each of the functions of exosomes depend on the exosome-releasing cell and the factor that stimulates exosome secretion [14]. In recent years, much attention has been paid to the use of exosomes in the treatment of diseases, and many studies have been conducted in this field. In this regard, these nanoparticles are used for the aim of drug delivery [15, 16]. A large part of blood exosomes are derived from platelets. They are released from platelets as a result of high shear stress, platelet activation under the influence of agonists, or apoptosis [17].

Historically, for the first time in 1967, platelet exosomes were observed using electron microscope and were called “platelet dust” [18]. In the following years, more studies were conducted on the structure and function of these particles derived from platelets. The studies showed that platelet exosomes have more specific procoagulation activity than activated platelets [19]. In recent years, it has been determined that platelet exosomes have many regeneration and repair properties in addition to pro-coagulation activity [20]. It seems that the function of platelet exosomes is due to the fact that they contain platelet growth factors that protect them from destruction and deliver them to the target cell [21].

It is very easy to prepare platelet exosomes and they can be prepared from Platelet-Rich plasma (PRP) by ultracentrifugation and ultrafiltration. They have a cup-like structure. Their surface is rich in exosome diagnostic markers such as CD9 , CD63, tumor susceptibility gene 101 (TSG101), and programmed cell death 6–interacting protein (PDCD6IP or ALIX). It also has platelet markers such as GPs GP IIb/IIIa, GP Ib/V/IX, CD62p, PF4 (CXCL4), pro-platelet basic protein (CXCL7) and cytoplasmic high-mobility group box 1 (HMGB1). [22-25]. Table 1 shows the structural features and storage conditions of platelet exosomes.

One of the important advantages of using platelet exosomes is their easy storage conditions. The most common storage method is freezing at -80 °C [26]. The number of vesicles and exosomes is stable in sodium citrate anticoagulation at room temperature. Also, the number of exosomes derived from platelets is stable in EDTA anticoagulation during 48 hours at room temperature [27].

Past studies have investigated the use of platelet-rich plasma in various branches of regenerative medicine and confirmed its usefulness but autologous platelets must be used in PRP therapy. Like PRP, platelet exosomes are rich in growth factors . Also, the use of platelet exosomes does not have side effects such as immunogenicity and tumorigenesis [21, 28]. Various studies have been conducted on the effectiveness and usefulness of exosomes derived from platelets in regenerative medicine, which we will review below. Table 2 and Figure 1 summarize the function of platelet exosomes in regenerative medicine.

Platelet exosomes extraction

The first step in using exosomes is to extract them. The gold standard method for extraction of exosomes is the Differential Ultracentrifugation (dUC), in which exosomes are separated based on size and density. One of the advantages of dUC is

Table 1. Structural features and storage conditions of platelet exosomes

size	30-100 nm
Surface markers	CD9 , CD63, TSG101, PDCD6IP or ALIX
Platelet markers	GP IIb/IIIa, GP Ib/V/IX, CD62p, PF4, CXCL7, HMGB1
Storage conditions	Freezing at -80°c

the absence of contamination with non-exosome proteins. Other methods include ultrafiltration, in which particles are separated based on their size in a short period of time without the need for much equipment. In immuno-based methods such as Immunoaffinity Capture, it is possible to identify and separate exosomes using specific antibodies against the exosomes surface markers. In these methods, the specificity and quality of the antibodies are very important, and it is also necessary to consume a large amount of antibodies [29-32].

Activating PRP with different agonists has a great effect on the concentration and quality of produced exosomes. For example, the use of calcium gluconate increases the concentration of obtained exosomes. Also, the use of thrombin agonist increases platelet growth factor and cytokines. The combination of thrombin and calcium gluconate enhance the concentration of exosomes, cytokines, and platelet growth factors [33]. Another study showed that the activation of PRP with calcium in the process of exosome production results in the production of exosomes that have a uniform shape and size, and the amount of contamination with other cellular components would be very low [34]. Recently, commercial kits containing Poly Ethylene Glycol (PEG) working based on the density difference of various particles are used by researchers [35]. It seems that the use of different techniques along with platelet activators is very effective in the quality of final exosomes, and more studies are needed in future in order to improve methods and techniques.

Platelet roles in angiogenesis

Platelets have an angiogenic role in tumors. In fact, the mediators in platelet granules have a dual anti-angiogenic and pro-angiogenic feature [36]. VEGF, bFGF, and PDGF have an angiogenic property, but PF4 has an anti-angiogenic role [37]. Recent studies have shown that platelets contain angiogenic bio-products and protect endothelial cells from damage. These findings drew attention to platelet-derived exosomes in regenerative medicine and angiogenesis. VEGF, bFGF, and PDGF of platelet microparticles increase angiogenesis in vitro through SRC kinase, PI3 kinase, and ERK signaling [38].

In addition to angiogenic factors, exosomes and platelet extracellular vesicles are also rich in small RNAs, and one of the most abundant of these RNAs are miRNAs, which have different roles in platelets. One of the most abundant miRNAs in exosomes and platelet extracellular vesicles is miR-126; it is a key regulator of angiogenesis and is known as an angiogenic miRNA that is abundantly found in endothelial cells and platelets. It has been shown that platelet vesicles by transferring miR-126 to the endothelial cells increase angiogenesis and the formation of tubular-like structures in these cells [39]. Therefore, it seems that the use of platelet exosomes is effective and efficient to induce angiogenesis and treatment of disorders such as ischemic heart diseases due to their content [40].

Hair repair

Hair is made of keratin and is located inside hair follicles. Every healthy man and woman has 80,000 to 120000 hairs on their scalp [41]. It has different roles, including protection from the sun's ultraviolet rays [42]. It also plays an important role in beauty and social acceptance. Normally, more than 100 strands of hair fall out every day, however, hair loss is one of the common problems around the world that many people are involved in and complain about it, and are looking for a solution to treat it [43]. Different clinical approaches are used to treat hair loss, such as drug therapy (for example Minoxidil [44], herbal extracts [45], platelet-rich plasma [46], and hair transplantation [47].

The growth of hair follicles is controlled by two groups of stem cells called mesenchymal and epithelial stem cells. Epithelial stem cells are

involved in the morphogenesis of hair follicles, while mesenchymal stem cells are located in structures called dermal papilla and directly causes the conversion of interfollicular keratinocytes of the skin into the follicular lineage [48, 49]. Therefore, dermal papilla has an important role in maintaining the function of hair follicles; it seems that new hair can be induced by dermal papilla and growth factors [50, 51].

In recent years, tissue engineering and regenerative medicine have made many efforts to induce dermal papilla in the treatment of hair loss, and extensive studies have been conducted in this field. It seems that studies based on exosomes have been effective in dermal papilla induction [52]. As mentioned above, one of the effective treatments for hair loss is the use of platelet-rich plasma.

Based on the presence of growth factors in the platelets, Nilforoushzadeh et al. investigated the effect of different concentrations [25, 50, 100 µg/ml] of Hair Outer Root Sheath Cells (HHORSCs –Exo) and Platelet-Lysis Exosomes (PL-Exo) to promote hair inductivity of dermal papilla cell. They investigated the rate of proliferation, migration, and the expression of alkaline phosphatase (ALP) (show the ability of papilla dermal cells to induce hair growth), versican, and α-SMA proteins (Dermal papilla cell identification markers) in papilla dermal cells after adding exosomes. The results showed that the concentration 100 µg/ml of HHORSCs –Exo significantly increased the proliferation, migration of dermal papilla, and expression of ALP, versican and α-SMA proteins compared to other test groups [53].

In another study, the effect of different concentrations (25, 50, 100 µg/ml) of Adipose-Derived Stem Cells and Platelet-Rich Plasma Exosomes on the induction of hair dermal papilla cells were investigated. These two groups of exosomes increased the proliferation and migration of dermal papilla cells due to the secretion of different growth factors such as (VEGF) and (PDGF), but the Adipose-Derived Stem Cells exosomes at a concentration of 100 µg/ml significantly increased the proliferation, migration of dermal papilla and expression of ALP, versican and α-SMA proteins and as a result hair induction compared to platelet-rich plasma exosomes [54]. Although the use of platelet exosomes such as PRP is effective in the treatment of hair loss, but more studies are still needed and perhaps it is necessary to use higher concentrations of platelet exosomes.

Wound healing

The term “chronic wounds” was first used in 1950 and refers to the wounds that are difficult to heal. Today, chronic wounds refer to wounds that take more than 3 months to heal and reduce the patient’s quality of life [55, 56]. Many factors such as diabetes, infections, arterial ischemia, malignant tumors, etc. cause such wounds [57].

Wound healing is a physiological process in which the normal function and structure of the tissue is restored. This process includes various stages such as homeostasis, inflammation, proliferation, and remodeling [58, 59]. Platelet growth factors cause angiogenesis as well as the production of Schwann cells and nerve regeneration [60]. Therefore, the use of platelets accelerates angiogenesis, epithelial regeneration, and wound healing.

Ischemic wound

In the studies conducted in ischemic wounds, it has been found that platelet extracellular vesicles stimulate angiogenesis in human umbilical vascular endothelial cells, as well as increase the proliferation and migration of fibroblast and keratinocyte cells. Transforming growth factor (TGF-β) is present in the extracellular vesicles of platelets and increases the expression of SMAD2, RAS, Mitogen-activated protein kinase kinase 3 (MKK3), RAS homolog family member A (RHOA), P38 ,Periostin, extracellular signal-regulated kinases 1 (ERK1), and Transforming growth factor beta-activated kinase 1(TAK1); as a result, it increases the secretion of collagen I and III, promote the differentiation of endothelial cells, and enhances fibroblast activation and wound healing. In the animal model of ischemic wounds, the combination of platelet exosomes and injectable surgical fibrin sealant (TISSEEL) causes rapid wound closure, skin regeneration, reduction of inflammation at the wound site, induction of hair follicles, and sebaceous glands at the wound site. Also, the activity of TGF-β and the increase in the expression of its downstream genes in TISSEEL-platelet exosomes are higher and cause an increase in the secretion of collagen I and III; as a result, it does not cause scarring at the site of wound healing [61].

Cutaneous wound

Re-epithelialization plays an important role in the cutaneous wound healing process, and ubiquitinization of proteins plays a reverse regulatory role in wound healing. Deubiquitinases

can remove the ubiquitin chain from a specific protein, stabilize it and accelerate re-epithelialization in the wound healing. The family of ubiquitin-specific proteases is one of the most important deubiquitinases, the most important member of which is USP15. EIF4A1 is also a key initiation factor in eukaryotes that is associated with the proliferation of various cell lines. It has been shown that the amount of USP15 is high in platelet exosomes, and also in wounds repaired with platelet exosomes, a high amount of USP15 is expressed; it seems that USP15 of platelet exosomes interacts with EIF4A1 causing wound healing [22].

Pressure ulcers

Pressure ulcers occur in thin elderly people, paralyzed people, and who have been hospitalized for a long time and cause damage to the skin and its underlying tissues in areas where there are bone protrusions or areas without fat cover. Spinal cord injury is an example of pressure ulcers, which has been shown to heal patients with platelets more effectively than using hydrogel [62]. In another study, platelets were used in patients with grade III pressure ulcers and hospitalized in addition to standard treatments, which led to a reduction in the volume of the wound, a reduction in the wound surface and a better recovery [63]. In a case report study, it was shown that dressing a pressure ulcer with platelet-rich fibrin weekly resulted in wound closure and healing after 4 weeks [64].

Diabetic wound

Other studies showed that platelet exosomes have a high concentration of growth factors without capsules and for this reason they can cause dephosphorylation and activation of Yes associated protein (YAP) through Rho-ROCK pathway. Then the active YAP goes to the nucleus and activates downstream factors. One of these factors is connective tissue growth factor (CTGF) that its activation increases the proliferation and migration of fibroblasts. On the other hand, the growth factors in exosomes activate the signaling pathway of PI3K/Akt and Erk. Activating this pathway increases angiogenesis at the cutaneous wound site in a diabetic rat model [65].

Sphingosine-1-phosphate is a vital bioactive lipid that plays a role in the regulation of angiogenesis and regeneration of blood vessels;it is present in a large amount in platelet exosomes. The receptors of this compound (S1PR1-3) are also expressed in the skin tissue. In mice models of diabetic wounds, the expression of S1PR1 increases. Therefore, the use of platelet exosomes accelerates wound healing process in these mice. It seems that S1P increases angiogenesis and diabetic wound healing through the signaling pathway of S1PR, p-AKT, FN1, and VEGF [66].

Gingival wound

In vitro studies on the healing of gingival wounds reported that the use of platelet extracellular vesicles increases wound closure and also increases the metabolic activity of fibroblast cells without cytotoxic effects. In the cells treated with these vesicles, the expression of MMP1 (Matrix metalloproteinase- 1) and COL1A1 (Collagen I α1) genes shows a significant increase, while no difference was observed in the expression of FN1 (Fibronectin) and VIM (Vimentin). The combination of hyaluronic acid (extracellular matrix glycosaminoglycan) with platelet extracellular vesicles increases metabolic activity and accelerates wound healing in the gum wounds. Also, the expression of fibronectin and vimentin genes decreases and the expression of MMP1 and COL1A1 increases. MMP1 is a collagen-degrading enzyme, while COL1A1 is an extracellular matrix compound, and the simultaneous increase of these proteins causes wound healing without scarring and fibrosis [67].

Eye injuries

One of the serious complications of diabetes is diabetic retinopathy, which can cause blindness. Platelet exosomes regulate inflammation by stimulating the production of reactive oxygen species (ROS) and malonylaldehyde and through the up regulation of the TLR4 signaling pathway. Also, CXCL10 derived from platelets causes retinal injury. In this way, platelet exosomes can play a role in the occurrence of diabetic retinopathy [68]. Another study showed that platelet exosomes isolated from diabetic rats with the activation of Yes-associated protein (YAP) cause the activation and promotion of connective tissue growth factor (CTGF) and the expression of fibronectin through the PI3K-Akt signaling pathway; as a result, they increase the activity of fibrogenic cells in human retinal Müller cells (hMCs)[69].

Corneal endothelial cells have very little ability

to regenerate themselves and are damaged under the influence of several factors such as aging. The in vitro studies show that treatment of corneal endothelial cells with platelet exosomes increases the viability, wound healing ability, and adhesion of cells [70].

Burn injury

Burns are common injuries caused by factors such as heat, electricity, and chemicals. Because of the complications of this kind of injury, such as bacterial infections, timely and effective treatment is very important. Treatment of burn mice models with mesenchymal stem cell exosomes and PRP increases VEGF and angiogenesis and epithelial regeneration. It also increases Bcl2, as well as reducing Bax and inflammation and oxidative damage at the wound site [71]. Another similar study showed that exosomes derived from mesenchymal stem cells and PRP reduce IL-6, IL-10, hyaluronidase, TGF-b, and MMP-3, and also decrease the expression of miRNA-203 (targeting the proliferative and migratory factors at the site of injury) and αSMA genes in the burn site; thus, they cause burn injury healing by anti-oxidant mechanisms [72].

Cosmetic surgery

The structure and special characteristics of exosomes have increased the interest of cosmetic surgeons in using these biological compounds in the treatment process. For example, one of the most common invasive methods in the treatment of skin aging complications and wounds is the use of fractional laser. So far, various methods have been used to reduce complications after laser. Recent studies have shown that the use of compounds and platelet exosomes will significantly reduce redness, pain, itching, peeling, bruising. Additionally, the skin of these people will be brighter and younger [73]. Using platelet exosomes for 4-6 weeks in facial injuries and skin aging improves skin health and rejuvenates it. This method is safe, tolerable, and liked by patients [74].

In intrinsic aging, the decrease in collagen production in the skin causes a decrease in the expression of connective tissue growth factor (GF), which is a regulator of collagen production. In rats suffering from intrinsic aging, treatment with platelet exosomes causes a decrease in the level of metalloproteinase 1 (MMP-1) and an increase in collagen, which indicates the ability of platelet exosomes to improve disease [75].

Orthopedic injuries

The movement system of the body includes bones, joints and skeletal muscles. It performs various functions such as movement and protection of vital organs. This system is susceptible to various acute and chronic injuries during life, such as orthopedic injuries. Although orthopedic injuries are more common in athletes, they can also be seen in other people. Due to poor blood supply and limited regeneration capacity in tendons, ligaments, and cartilages the healing process in these tissues is slow. Also, the healing process in the injury articular cartilage is not satisfactory and leads to complications such as osteoarthritis.

Muscular injuries are also very common in athletes, which causes gradual disability. Bone injuries are caused by various factors such as trauma, tumor, necrosis, infection, and fractures. One of the challenges related to this injury is the limited regeneration capacity of bone tissue, which can eventually cause osteoarthritis, osteomyelitis, and chronic wounds. Ineffectiveness of traditional treatments have led to turning to alternative treatments. One of these treatments is the use of regenerative medicine via platelets [76].

The use of cell therapy in the repair of muscle damage has been proven. Recent studies have shown that mesenchymal stem cell exosomes and platelet exosomes are very beneficial in repairing muscle damage and returning normal muscle function. In repaired models by platelet exosomes, Myogenin, which is a muscle regulatory factor and an indicator of myogenesi, showed up-regulation; while in muscles repaired with mesenchymal stem cell-derived exosomes, TGF-beta was significantly reduced, which is a key factor in muscle recovery by regulating the reset of the extracellular matrix and inflammation. These exosomes repair muscle damage by modulating the inflammation, fibrosis, and myogenesis [77].

One of the most common musculoskeletal disorders is tendonopathy with a prevalence of 30-50%, which remains an unresolved inflammatory problem. In an effort to find a cure for this disorder, researchers found that platelet-derived vesicles and exosomes can be used as a strong and effective treatment by increasing the expression of tendonogenic markers, extracellular matrix regeneration, and the synthesis of

anti-inflammatory mediators [78]. Another common injury is sprained ankle, which can cause frequent sprains and instability of the ankle and finally the occurrence of osteoarthritis (OA). In order to find an effective treatment for this condition, researchers used platelet exosomes. Platelet exosomes are rich in SDF1 (CXCL12), which, after being released, bind to their receptor,CXCR4, on the surface of bone mesenchymal stem cells and cause them to be absorbed at the site of injury and repair the OA [79].

The mRNA expression of collagen II, aggrecan, and SOX2, which are chondrogenic differentiation factors increases in cells treated with platelet exosomes. TGF-β found in exosomes induces cartilage differentiation by activating the signaling pathways of Smad2/3, ERK1/2 and p38. Inflammatory cytokines such as IL-1β and TNF-α play an important role in the occurrence of osteoarthritis and cartilage destruction. Platelet exosomes reverse the inflammatory effect of these cytokines by reducing the activation of NF-κB [79]. Recent studies have shown that the wnt/β-catenin signaling pathway plays a key role in the pathogenesis of osteoarthritis, and the downstream mediators and effectors are increased in osteoarthritis.

Various studies conducted on humans or animals showed that the wnt/ β-catenin pathway plays a role in bone and joint pathology with a direct effect on bone, cartilage, and synovial fluid. Therefore, it is possible to reduce these pathological effects by targeting this path [80]. Invitro models of osteoarthritis confirmed increased expression of β-catenin, wnt5A, and RUNX2, which are the downstream effector proteins of this pathway. It means that the wnt/β-catenin signaling pathway is activated and causes osteoarthritis. In contrast, platelet exosomes in addition to reducing inflammatory cytokines and increasing the proliferation and migration of chondrocytes, causes the reduction of these proteins and thereby reduces osteoarthritis [81]. While other studies showed that in osteonecrosis caused by corticosteroids platelet exosomes increase the level of RUNX2, β catenin, and collagen II; they are osteogenesis factors.

Corticosteroids cause the activation of ER stress (endoplasmic reticulum) and finally the activation of PERK (protein kinase RNA-like ER kinase). CHOP (CCAAT-enhancer-binding protein homologous protein) is an effective protein in the downstream of PERK and is activated and inhibits the expression of BCL-2 (B-cell lymphoma 2). Then caspase 3 is cleaved and cell apoptosis occurs. This is while platelet exosomes prevent PERK activation and CHOP expression by increasing the expression of BCL-2.

Akt is a strong signal that inhibits apoptosis, which is activated under the influence of platelet exosomes. By activating the Akt, pro-inflammatory mediators such as Bad are inhibited, the expression of BCL-2 increases and finally caspase 3 is also inhibited. In this way, platelet exosomes prevent bone cell apoptosis and osteonecrosis through the activation of the Akt/Bad/BCL-2 signaling pathway. Also, platelet exosomes contains factors such as VEGF, bFGF, and PDGF in a large amount can easily activate the Akt and ERK signal. In this way, they can cause angiogenesis and protect bone blood vessels against osteonecrosis caused by corticosteroids [28].

Table 2. A summary of the function of platelet exosomes in regenerative medicine

	Signaling	Advantage	Reference
Hair Repair	↑ ALP , versican and α-SMA	↑proliferation, migration of dermal papilla and maintaining the hair follicles function	41,42
Wound healing	SMAD2/ Periostin, TAK1/p38, RAS/ERK, MKK3, Rho-ROCK/YAP, PI3K/Akt	collagen I and III synthesis, endothelial cells differentiation, ↑CTGF and fibroblast activation,↑ angiogenesis	49,50,51
Orthopedic injuries	CXCL12/CXCR4, Smad2/3, ERK1/2 , p38,inhibition NF-κB, inhibition wnt/β-catenin , Akt/Bad/BCL-2	OA repair, cartilage differentiation, Inflammatory cytokines inhibition, inhibits apoptosis, ↑ chondrogenic differentiation factors	55,57,28
Angiogenesis	SRC kinase, PI3 kinase, ERK, miR-126	↑angiogenesis and the formation of VWF-positive small vessels	60,61
Drug delivery	PTX-EX, Dox-EX, , Lamivudine –EX ,Tenofovir-EX	preventing angiogenesis, cell migration and invasion, treatment of breast cancer, lower dose of the drug will be needed, ↑toxicity of the drug on leukemic cells , slow release of the drug at the site of action	66,67,68

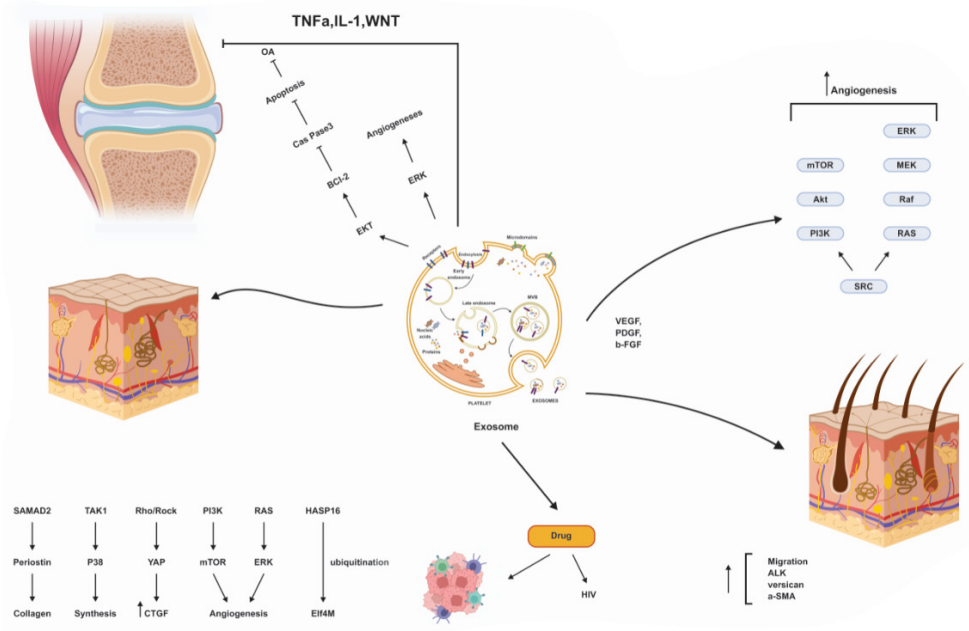

Fig.1. Role of platelet exosomes in regenerative medicine. Platelet growth factors present in exosomes cause hair repair by increasing the expression of ALP, versican and α-SMA. By stimulating the ERK and PI3K signaling pathway, they increase angiogenesis. By inhibiting inflammatory cytokines and wnt/β-catenin pathway and prevention of apoptosis prevents the occurrence of osteoarthritis. By affecting the signaling pathways of SMAD2, TAK1, Rho/Rock, PI3K, RAS and participating in the ubiquitination of Elf4M, it causes wound healing. Also, due to its special structure, it is very efficient in drug delivery.

Other applications of platelet derived exosome except regenerative medicine

Drug delivery

Conventional methods of using drugs have many limitations such as limited effectiveness, low solubility, lack of selectivity, drug aggregation, and low bioavailability. These limitations can be overcome using a drug delivery system. The drug delivery system includes methods that transfer the drug, release and absorb it at the target site. It prevents the unwanted effects of the drug on the vital organs of the body. It also prevents rapid clearance and increases the concentration of the drug in the target tissue; for this reason, lower doses of the drug will be needed [82, 83].

As mentioned, platelet exosomes have a bi-layer membrane made of phospholipids. For this reason, they can protect their internal compounds and transfer them to different places. This characteristic has attracted the scientists attention to be used as drug delivery system [84]. One of the uses of drug delivery system is to transfer chemotherapy drugs, which have many side effects for vital organs. Paclitaxel (PTX) is an anti-cancer drug that is used in the treatment of breast cancer. Targeting breast cancer cells with PTX-EX has therapeutic effects such as preventing angiogenesis, preventing cell migration and invasion [85]. Doxorubicin (Dox) is used in the treatment of breast cancer; in order to reduce the toxicity of Dox on other cells, it can be loaded into platelet exosomes.

It has been shown that the Dox transfer by platelet exosomes to cancer cells causes a higher concentration of the drug in the desired location compared to the drug alone. Therefore, a lower dose of the drug will be needed, and the drug toxicity is higher in cancer cells [86].

Acquired Immune Deficiency Syndrome (HIV) is a viral disease whose treatment is one of the biggest global challenges. The use of antiviral drugs has many limitations. The plasma membrane is one of the major barriers in the use of antiviral drugs that cause the spread of HIV. Also, the side effects of these drugs have led to the use of drug delivery system. Loading of the two antiviral drugs of Lamivudine and Tenofovir in platelet micro particles increases the inhibitory effect on the HIV-1 virus, the slow release of the drug at the site of action, and also reducing the toxicity on the organs [87].

Tumor metastasis is responsible for many mortality in colorectal cancer. Although the mechanism of metastasis is not well known,

recently it has been shown that epithelial-mesenchymal transition (EMT) plays an important role in the pathogenesis of carcinogenic tumor metastasis. During the EMT process, epithelial cells lose their polarity and cell-cell adhesion by decreasing the expression of E-cadherin, and by increasing the expression of transcription factors such as ZEB1, ZEB2, Vimentin, N-cadherin, Snail, Slug, and Twist has characteristics of mesenchymal stem cell, such as invasion and migration. Past studies show that microRNAs such as miR-375-3p have tumor suppression properties and prevent the migration of tumor cells. Therefore, Rezaei et al. investigated the effect of Exosomes containing mir -375-3p on colon cancer cell lines (HT-29 and SW-480) by loading miR-375-3p in tumor-derived exosomes. The results of this study showed that mir-375-3p was effectively delivered by exosomes and caused an increase in the expression of E-cadherin and a decrease in the expression of β-catenin, vimentin, ZEB1, and snail. Also, the migration and invasion ability of HT-29 and SW 480-480 was inhibited by these exosomes and the amount of CD44 and CD133 which increases in EMT also decreased in these cells. The results of this research showed that exosomes containing Mir-375 are strong therapeutic elements for the treatment of colorectal cancer [88].

Shams et al. have used platelet derived exosomes as a carrier to deliver miRs. They transferred miR-150 in to the megakaryocytic cell line of M07-e and successfully altered c-myb gene expression; it is a target gene of the cited miR. They stated that the loaded cargo released over time; the amount of released miR-150 in the cell line was higher after 48 hours incubation compared to 24 hours. This finding (slow release) could be an important feature in the drug delivery system. They aimed to induce cell cycle transition but they could not to do it; it seems that the interaction of the loaded cargo with exosome content leads to failure in mitosis [89]. Also, they introduced and compared different methods to isolate platelet derived exosomes [90]. The conducted investigations showed that the use of platelet exosomes in the drug delivery system is a highly efficient method in the treatment of various diseases.

Cardiovascular disease

Atherosclerosis is known as a chronic inflammatory disease that is associated with low-density lipoproteins. Also, human coronary vascular endothelial cells (CVECs) activated by inflammation play a role in the development of atherosclerosis. A study showed that the platelet exosomes of ApoE−/− mouse models with atherosclerosis have a high expression of miR-25-3p, and CVECs endocytose platelet exosomes also increase the expression of miR-25-3p; the presence of these exosomes reduces the expression of inflammatory cytokines and inhibits the NF-κB signaling pathway and targets Adam10 (a metalloprotease effective in inflammation) and reduces its expression [91].

Platelet exosomes activated with thrombin are rich in miR-223, miR-339, and miR-21 which reduce the expression of platelet-derived growth factor receptor-beta (PDGFRβ), and by entering to the vascular smooth muscles, prevents their proliferation caused by PDGF stimulation cells. In this way, these exosomal microRNAs can be a predictive biomarker of atherothrombosis [92].

Chronic inflammation plays a vital role in the pathophysiology of coronary artery diseases. It has been shown that platelet exosomes contain a large amount of microRNA-34c-5p, which by targeting podocalyxin (PODXL) and inhibiting the P38 MAPK signaling pathway, reduces the expression of inflammatory cytokines and prevents the occurrence of inflammation in coronary artery endothelial cells [93]. In this way, it seems that the compounds in platelet exosomes, especially microRNAs, play a significant role in improving cardiovascular diseases.

Neurodegenerative disorders

One of the common treatments for intracranial aneurysm is the use of endovascular coils, which is associated with the risk of disease recurrence and the need for repeated treatment. In a study, researchers showed that covering platinum coils with platelet exosomes increases the attachment and proliferation of endothelial cells without thrombogenic risks. It seems that this treatment method is very safe and useful in aneurysm healing [94].

Due to the disabilities caused by stroke, efforts to find effective treatment continue. Studies in mouse models of stroke show that the use of platelet microparticles such as exosomes increases cell proliferation, angiogenesis and neurogenesis in brain cells. In this way, it improves brain function and behavior [95].

Parkinson's is a movement disorder that can eventually lead to behavioral disorder and dementia. Increased amyloid β 1-42 is associated with dementia. It has been shown that platelet exosomes in patients with Parkinson's disease and dementia are rich in Aβ 1-42. Accordingly, the use of platelet exosomes and their Aβ 1-42 measurement as a diagnostic and prognostic marker in patients with the Parkinson's disease dementia [96].

Diagnosis and prognostic applications:

In ovarian cancer, platelets by accumulating and activating in ascites fluid can cause cancer progression. In a study, it was shown that the progression of cancer can be estimated by quantitative measurement of platelet exosomes in ascites [97].

Breast cancer is one of the most common malignancies in women. It has been found that platelet exosomes play a significant role in the progression of this cancer. Exosomes from activated platelets increase the expression of vimentin, fibronectin, Snai1, and N-cadherin in cancer cells; in this way, they help the invasion of disease [98].

Neutrophils play a vital role in the severity of sepsis and septic shock through netosis. In the culture of neutrophils with platelet exosomes of healthy people and people with sepsis, it was observed that the rate of netosis is significantly high in neutrophils cultured with exosomes of sepsis individuals. Also, in animal models of septic shock with platelet depletion, the amount of exosomes, netosis, and lung damage was greatly reduced. It seems that platelet exosomes through exosomal high-mobility group protein 1 (HMGB1) and/or miR-15b-5p and miR- 378a-3p cause the induction of netosis and damages [99].

In recent years, much attention has been paid to the role of platelet exosomes in the diagnosis of various diseases, including Alzheimer's. Recently, scientists have focused on diagnosing mild cognitive disorders (MCD) in the early stages and preventing its progression to Alzheimer's. It has been found in patients with MCD, who are amyloid β peptide positive (associated with the progression of Alzheimer's), the expression of miR-1233-5p in platelet exosomes decreases. The reduction of this microRNA increases the expression of p-selectin and the adhesion of platelets to fibronectin through p-selectin that was caused by amyloidβ peptide. The measurement of microRNAs in platelet exosomes plays a role in diagnosing and predicting progression to Alzheimer's disease [100].

The above cases are examples of the role of platelet derived exosomes in the diagnosis and prognosis of different diseases.

Clinical trial

Many clinical trials have been conducted in the use of various exosomes, such as mesenchymal stem cell exosomes in the treatment of COVID-19 pneumonia or cartilage repair, and their efficacy has been proven to some extent in humans [101-103]. However, clinical trial studies in platelet exosomes have been very limited, and we find only one study that are described below.

Efforts to treat wounds, especially chronic wounds that do not respond to treatment are always ongoing. Due to the effectiveness of platelet exosomes in wound healing in vitro, the first clinical trial study was conducted for this purpose on healthy volunteers. In a double-blind study, Allogeneic platelets were extracted by Ligand-based Exosome Affinity Purification (LEAP) chromatography by Gregor Lichtfuss and colleagues, and the quality and sterility of the isolated exosomes were checked. In the participants of the study, after the examination two wounds were created by a skin biopsy punch in the inner arm area. In each person, one wound was treated with subcutaneous injection of platelet exosome, and the other wound was treated with subcutaneous injection of placebo. With the 30-day follow-up of the participants, it was found that the injection of exosome was not associated with serious complications or death, and the wound healed well without scarring [104].

Limitations

In recent years, a lot of attention has been paid to the use of platelet exosomes in the treatment of various diseases and regenerative medicine. But in addition to its many benefits, the use of these exosomes has limitations that are briefly stated here. So far, researchers have used many methods to extract platelet exosomes, but there is still no standard method and a reference technique for extracting exosomes. For this reason, it is not possible to distinguish between different methods and the quantity and quality of the obtained exosomes [23].

Like living cells, apoptotic cells can also produce exosomes that are associated with therapeutic exosomes and interfere with the treatment process. Due to the induction of inflammatory cytokine production by macrophages, these exosomes have a great role in the occurrence of inflammation, specific immune responses, or suppression of the immune system. They can also play a role in the development of cancer or inflammatory diseases [105]. These cases have limited the use of exosomes.

CONCLUSION

In this review study, we briefly studied the role of platelet exosomes in the regenerative medicine. Platelet exosomes have the ability to store and transport important biomaterial such as the compounds found in platelet granules, as well as drugs. For this reason, they have received much attention in regenerative medicine. Platelet exosomes play a very strong and important role in various branches of regenerative medicine, such as wound healing and angiogenesis, nerve cell regeneration, musculoskeletal damage repair, and hair loss treatment. Although the use of platelet exosomes seems to be a safe and effective method, more studies are needed to evaluate the effects of this therapeutic method in other fields of regenerative medicine and disease treatment, and to answer many unanswered questions in this field.

ACKNOWLEDGMENTS

The authors wish to thank all our colleagues in High Institute for Research and Education in Transfusion Medicine.

FUNDING

This review article received no funds.

DATA AVAILABILITY

All the data have been inlcuded in the manuscript and will be made available upon publication of the manuscript.

ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Mason C, Manzotti E. Regenerative medicine cell therapies: numbers of units manufactured and patients treated between 1988 and 2010. Regen Med. 2010;5(3):307-313.
2. Yu Y, Wang Q, Wang C, Shang L. Living materials for regenerative medicine. Engineered Regeneration. 2021;2:96-104.
3. Sampogna G, Guraya SY, Forgione A. Regenerative medicine: Historical roots and potential strategies in modern medicine. J Microsc Ultrastruct. 2015;3(3):101-107.
4. Fritsma GA. Platelet structure and function. Clin Lab Sci. 2015;28(2):125-131.
5. van der Meijden PE, Heemskerk JW. Platelet biology and functions: new concepts and clinical perspectives. Nat Rev Cardiol. 2019;16(3):166-179.
6. Emer J. Platelet-rich plasma (PRP): current applications in dermatology. Skin Therapy Lett. 2019;24(5):1-6.
7. Lubkowska A, Dolegowska B, Banfi G. Growth factor content in PRP and their applicability in medicine. J Biol Regul Homeost Agents. 2012;26(2 Suppl 1):3S-22S.
8. Oneto P, Etulain J. PRP in wound healing applications. Platelets. 2021;32(2):189-199.
9. Davis ME. Exosomes: what do we love so much about them? Circ Res. 2016;119(12):1280-1282.
10. Sharma S, Rasool HI, Palanisamy V, Mathisen C, Schmidt M, Wong DT, et al. Structural-mechanical characterization of nanoparticle exosomes in human saliva, using correlative AFM, FESEM, and force spectroscopy. ACS Nano. 2010;4(4):1921-1926.
11. Ha D, Yang N, Nadithe V. Exosomes as therapeutic drug carriers and delivery vehicles across biological membranes: current perspectives and future challenges. Acta Pharm Sin B. 2016;6(4):287-296.
12. Suchorska WM, Lach MS. The role of exosomes in tumor progression and metastasis. Oncol Rep. 2016;35(3):1237-1244.
13. Qin J, Xu Q. Functions and application of exosomes. Acta Pol Pharm. 2014;71(4):537-543.
14. Van Niel G, d'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. Nat Rev Mol Cell Biol. 2018;19(4):213-228.
15. Haney MJ, Klyachko NL, Zhao Y, Gupta R, Plotnikova EG, He Z, et al. Exosomes as drug delivery vehicles for Parkinson's disease therapy. J Control Release. 2015;207(July).18-30.
16. Liang Y, Duan L, Lu J, Xia J. Engineering exosomes for targeted drug delivery. Theranostics. 2021;11(7):3183-3195.
17. Aatonen M, Grönholm M, Siljander PR. Platelet-derived microvesicles: multitalented participants in intercellular communication. Semin Thromb Hemost. 2012; 38(1):102-113.
18. Wolf P. The nature and significance of platelet products in human plasma. Br J Haematol. 1967;13(3):269-288.
19. Sinauridze EI, Kireev DA, Popenko NY, Pichugin AV, Panteleev MA, Krymskaya OV, et al. Platelet microparticle membranes have 50-to 100-fold higher specific procoagulant activity than activated platelets. Thromb Haemost. 2007;97(03):425-434.
20. Wu J, Piao Y, Liu Q, Yang X. Platelet-rich plasma-derived extracellular vesicles: A superior alternative in regenerative medicine? Cell Prolif. 2021;54(12):e13123.
21. Torreggiani E, Perut F, Roncuzzi L, Zini N, Baglìo S, Baldini N. Exosomes: novel effectors of human platelet lysate activity. Eur Cell Mater. 2014;28:137-151.
22. Xu Y, Lin Z, He L, et al. Platelet-Rich Plasma-Derived Exosomal USP15 Promotes Cutaneous Wound Healing via Deubiquitinating EIF4A1. *Oxid Med Cell Longev*. 2021; 1:9674809.

23. Spakova T, Janockova J, Rosocha J. Characterization and therapeutic use of extracellular vesicles derived from platelets. Int J Mol Sci. 2021;22(18):9701.
24. Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J Extracell Vesicles. 2018;7(1):1535750.
25. Antwi-Baffour S, Adjei J, Aryeh C, Kyeremeh R, Kyei F, Seidu MA. Understanding the biosynthesis of platelets-derived extracellular vesicles. Immun Inflamm Dis. 2015;3(3):133-140.
26. Coumans FA, Brisson AR, Buzas EI, Dignat-George F, Drees EE, El-Andaloussi S, et al. Methodological guidelines to study extracellular vesicles. Circ Res. 2017;120(10):1632-1648.
27. Wisgrill L, Lamm C, Hartmann J, Preißing F, Dragosits K, Bee A, et al. Peripheral blood microvesicles secretion is influenced by storage time, temperature, and anticoagulants. Cytometry A. 2016;89(7):663-672.
28. Tao S-C, Yuan T, Rui B-Y, Zhu Z-Z, Guo S-C, Zhang C-Q. Exosomes derived from human platelet-rich plasma prevent apoptosis induced by glucocorticoid-associated endoplasmic reticulum stress in rat osteonecrosis of the femoral head via the Akt/Bad/Bcl-2 signal pathway. Theranostics. 2017;7(3):733-750.
29. Sidhom K, Obi PO, Saleem A. A review of exosomal isolation methods: is size exclusion chromatography the best option?. Int J Mol Sci. 2020;21(18):6466.
30. Livshits MA, Khomyakova E, Evtushenko EG, Lazarev VN, Kulemin NA, Semina SE, et al. Isolation of exosomes by differential centrifugation: Theoretical analysis of a commonly used protocol. Sci Rep. 2015;5(1):17319.
31. Konoshenko MY, Lekchnov EA, Vlassov AV, Laktionov PP. Isolation of Extracellular Vesicles: General Methodologies and Latest Trends. *Biomed Res Int*. 2018; 2018:8545347.
32. Koliha N, Wiencek Y, Heider U, Jüngst C, Kladt N, Krauthäuser S, et al. A novel multiplex bead-based platform highlights the diversity of extracellular vesicles. J Extracell Vesicles. 2016;5(1):29975.
33. Rui S, Yuan Y, Du C, Song P, Chen Y, Wang H, et al. Comparison and investigation of exosomes derived from platelet-rich plasma activated by different agonists. Cell Transplant. 2021;30:09636897211017833.
34. Saumell-Esnaola M, Delgado D, García del Caño G, Beitia M, Sallés J, González-Burguera I, et al. Isolation of platelet-derived exosomes from human platelet-rich plasma: Biochemical and morphological characterization. Int J Mol Sci. 2022;23(5):2861.
35. Yakubovich E, Polischouk A, Evtushenko V. Principles and problems of exosome isolation from biological fluids. Biochem (Mosc) Suppl Ser A Membr Cell Biol. 2022;16(2):115-126.
36. Pinedo H, Verheul H, D'amato R, Folkman J. Involvement of platelets in tumour angiogenesis? . Lancet. 1998;352(9142):1775-1777.
37. Brill A, Elinav H, Varon D. Differential role of platelet granular mediators in angiogenesis. Cardiovasc Res. 2004;63(2):226-235.
38. Brill A, Dashevsky O, Rivo J, Gozal Y, Varon D. Platelet-derived microparticles induce angiogenesis and stimulate post-ischemic revascularization. Cardiovasc Res. 2005;67(1):30-38.
39. Bordin A, Chirivì M, Pagano F, Milan M, Iuliano M, Scaccia E, et al. Human platelet lysate-derived extracellular vesicles enhance angiogenesis through miR-126. Cell Prolif. 2022;55(11):e13312.
40. Li Q, Song Y, Wang Q, Chen J, Gao J, Tan H, et al. Engineering extracellular vesicles with platelet membranes fusion enhanced targeted therapeutic angiogenesis in a mouse model of myocardial ischemia reperfusion. Theranostics. 2021;11(8):3916-3931.41. Paus R, Cotsarelis G. The biology of hair follicles. N Engl J Med. 1999; 341(7):491-497.
42. Cash T. The psychosocial consequences of androgenetic alopecia: a review of the research literature. Br J Dermatol. 1999;141(3):398-405.
43. Wolff H, Fischer TW, Blume-Peytavi U. The diagnosis and treatment of hair and scalp diseases. Dtsch Arztebl Int. 2016;113(21):377-386.
44. Randolph M, Tosti A. Oral minoxidil treatment for hair loss: a review of efficacy and safety. J Am Acad Dermatol. 2021;84(3):737-746.
45. Zgonc Škulj A, Poljšak N, Kočevar Glavač N, Kreft S. Herbal preparations for the treatment of hair loss. Arch Dermatol Res. 2020;312:395-406.
46. Badran KW, Sand JP. Platelet-rich plasma for hair loss: review of methods and results. Facial Plast Surg Clin North Am. 2018;26(4):469-485.
47. Ekelem C, Pham C, Mesinkovska NA. A systematic review of the outcome of hair transplantation in primary scarring alopecia. Skin Appendage Disord. 2019;5(2):65-71.
48. Kalabusheva E, Terskikh V, Vorotelyak E. Hair Germ Model In Vitro via Human Postnatal Keratinocyte-Dermal Papilla Interactions: Impact of Hyaluronic Acid. Stem Cells Int. 2017;2017:9271869.
49. Bukvić Mokoš Z, Lazić Mosler E. Advances in a rapidly emerging field of hair follicle stem cell research. Coll Antropol. 2014;38(1):373-378.
50. Yang C-C, Cotsarelis G. Review of hair follicle dermal cells. J Dermatol Sci. 2010;57(1):2-11.
51. Zhu N, Huang K, Liu Y, et al. miR-195-5p Regulates Hair Follicle Inductivity of Dermal Papilla Cells by Suppressing Wnt/*β*-Catenin Activation. *Biomed Res Int*. 2018; 2018:4924356.
52. Phinney DG, Pittenger MF. Concise review: MSC-derived exosomes for cell-free therapy. Stem cells. 2017;35(4):851-858.
53. Nilforoushzadeh MA, Aghdami N, Taghiabadi E. Human hair outer root sheath cells and platelet-lysis exosomes promote hair inductivity of dermal papilla cell. Tissue Eng Regen Med. 2020;17(4):525-536.
54. Nilforoushzadeh MA, Aghdami N, Taghiabadi E. Effects of Adipose-Derived Stem Cells and Platelet-Rich Plasma Exosomes on The Inductivity of Hair Dermal Papilla Cells. Cell J. 2021; 23(5):576-583.
55. Kyaw B, Jaerbrink K, Martinengo L, Car J, Harding K, Schmidtchen A. Need for improved definition of "chronic wounds" in clinical studies. Acta Derm Venereol. 2017;98(1):157-158.
56. Sen CK. Human Wounds and Its Burden: An Updated Compendium of Estimates. Adv Wound Care (New Rochelle). 2019; 8(2):39-48.
57. Morton LM, Phillips TJ. Wound healing and treating wounds: Differential diagnosis and evaluation of chronic wounds. J Am Acad Dermatol. 2016;74(4):589-605.
58. PC S. Gingival Wound Healing. J Dent Res. 2014;94(3):395-402.
59. Etulain J. Platelets in wound healing and regenerative medicine. Platelets. 2018;29(6):556-568.
60. Wu P, Tong Z, Luo L, Zhao Y, Chen F, Li Y, et al.

Comprehensive strategy of conduit guidance combined with VEGF producing Schwann cells accelerates peripheral nerve repair. Bioact Mater. 2021;6(10):3515-3527.
61. Shi A, Li J, Qiu X, et al. TGF-β loaded exosome enhances ischemic wound healing *in vitro* and *in vivo*. Theranostics. 2021; 11(13):6616-6631.
62. Singh G, Borah D, Khanna G, Jain S. Efficacy of local autologous platelet-rich plasma in the treatment of pressure ulcer in spinal cord injury patients. Cureus. 2021;13(10):e18668.
63. Habib TN, Fayed AM, Deghady AA, Maklad OM, Ahmed IE. Autologous platelet-rich plasma in the treatment of third-degree pressure ulcers; pilot randomized-controlled trial. Research and Opinion in Anesthesia & Intensive Care. 2023;10(3):236-241.
64. Swarnakar R, Rahman H, Venkataraman S. "Platelet-Rich Fibrin Membrane-as a novel biomaterial for pressure injury healing in a person with spinal cord injury: A case report". *Spinal Cord Ser Cases*. 2022; 8(1):75.
65. Guo SC, Tao SC, Yin WJ, Qi X, Yuan T, Zhang CQ. Exosomes derived from platelet-rich plasma promote the re-epithelization of chronic cutaneous wounds via activation of YAP in a diabetic rat model. *Theranostics*. 2017; 7(1):81-96.
66. Chen T, Song P, He M, et al. Sphingosine-1-phosphate derived from PRP-Exos promotes angiogenesis in diabetic wound healing via the S1PR1/AKT/FN1 signalling pathway. *Burns Trauma*. 2023; 11:tkad003.
67. Antich-Rosselló M, Munar-Bestard M, Forteza-Genestra MA, Calvo J, Gayà A, Monjo M, et al. Platelet Derived Extracellular Vesicles Promote in Vitro Gingival Wound Healing. 2021 (preprint).
68. Zhang W, Dong X, Wang T, Kong Y. Exosomes derived from platelet-rich plasma mediate hyperglycemia-induced retinal endothelial injury via targeting the TLR4 signaling pathway. *Exp Eye Res*. 2019; 189:107813.
69. Zhang W, Jiang H, Kong Y. Exosomes derived from platelet-rich plasma activate YAP and promote the fibrogenic activity of Müller cells via the PI3K/Akt pathway. Exp Eye Res. 2020;193:107973.
70. Widyaningrum R, Wu Y-W, Delila L, Lee D-Y, Wang T-J, Burnouf T. In vitro evaluation of platelet extracellular vesicles (PEVs) for corneal endothelial regeneration. Platelets. 2022;33(8):1237-1250.
71. Tammam BM, Habotta OA, El-Khadragy M, Moneim AEA, Abdalla MS. Therapeutic role of mesenchymal stem cells and platelet-rich plasma on skin burn healing and rejuvenation: A focus on scar regulation, oxido-inflammatory stress and apoptotic mechanisms. Heliyon. 2023;9(9):e19452.
72. Imam R, Amer M. Potential therapeutic role of microvesicles derived from mesenchymal stem cells and platelet-rich plasma in murine burn wound healing: scar regulation and antioxidant mechanism. *Folia Morphol (Warsz)*. 2023;82(3):656-667.
73. Dayan S, Gandhi N, Wilson J, Kola E, Jankov LE, Copeland K, et al. Safety and efficacy of human platelet extract in skin recovery after fractional CO2 laser resurfacing of the face: A randomized, controlled, evaluator-blinded pilot study. J Cosmet Dermatol. 2023;22(9):2464-2470.
74. Proffer SL, Paradise CR, DeGrazia E, Halaas Y, Durairaj KK, Somenek M, et al. Efficacy and tolerability of topical platelet exosomes for skin rejuvenation: six-week results. Aesthet Surg J. 2022;42(10):1185-1193.
75. Rosmarwati E, Ellistasari EY, Kusumawardani A, Julianto I, Widhiati S, Setyawan NA, et al. Human platelet lysate-derived exosomes are superior to the lysate at increasing collagen deposition in a rat model of intrinsic aging. Journal of Applied Pharmaceutical Science. 2023; 13(6):211-216.
76. Fang J, Wang X, Jiang W, Zhu Y, Hu Y, Zhao Y, et al. Platelet-rich plasma therapy in the treatment of diseases associated with orthopedic injuries. Tissue Eng Part B Rev. 2020;26(6):571-585.
77. Iyer SR, Scheiber AL, Yarowsky P, Henn III RF, Otsuru S, Lovering RM. Exosomes isolated from platelet-rich plasma and mesenchymal stem cells promote recovery of function after muscle injury. *Am J Sports Med* . 2020;48(9):2277-2286.
78. Graça AL, Domingues RM, Calejo I, Gómez-Florit M, Gomes ME. Therapeutic effects of platelet-derived extracellular vesicles in a bioengineered tendon disease model. Int J Mol Sci. 2022;23(6):2948.
79. Zhang Y, Wang X, Chen J, Qian D, Gao P, Qin T, et al. Exosomes derived from platelet-rich plasma administration in site mediate cartilage protection in subtalar osteoarthritis. J Nanobiotechnology. 2022;20(1):1-22.
80. Zhou Y, Wang T, Hamilton JL, Chen D. Wnt/β-catenin signaling in osteoarthritis and in other forms of arthritis. Curr Rheumatol Rep. 2017;19:1-8.
81. Liu X, Wang L, Ma C, Wang G, Zhang Y, Sun S. Exosomes derived from platelet-rich plasma present a novel potential in alleviating knee osteoarthritis by promoting proliferation and inhibiting apoptosis of chondrocyte via Wnt/β-catenin signaling pathway. J Orthop Surg Res. 2019;14(1):1-16.
82. Wilczewska AZ, Niemirowicz K, Markiewicz KH, Car H. Nanoparticles as drug delivery systems. Pharmacol Rep. 2012;64(5):1020-1037.
83. Li C, Wang J, Wang Y, Gao H, Wei G, Huang Y, et al. Recent progress in drug delivery. Acta Pharm Sin B. 2019;9(6):1145-1162.
84. Heidarzadeh M, Gürsoy-Özdemir Y, Kaya M, Eslami Abriz A, Zarebkohan A, Rahbarghazi R, et al. Exosomal delivery of therapeutic modulators through the blood–brain barrier; promise and pitfalls . Cell Biosci. 2021;11:1-28.
85. Meliciano A, Salvador D, Mendonça P, Louro AF, Serra M. Clinically Expired Platelet Concentrates as a Source of Extracellular Vesicles for Targeted Anti-Cancer Drug Delivery. Pharmaceutics. 2023;15(3):953.
86. Uslu D, Abas BI, Demirbolat GM, Cevik O. Effect of platelet exosomes loaded with doxorubicin as a targeted therapy on triple-negative breast cancer cells. *Mol Divers*. 2024; 28(2):449-460.
87. Soleymani S, Yari F, Bolhassani A, Bakhshandeh H. Platelet microparticles: An effective delivery system for anti-viral drugs. J Drug Deliv Sci Technol. 2019;51(June):290-296.
88. Rezaei R, Baghaei K, Amani D, Piccin A, Hashemi SM, Aghdaei HA, et al. Exosome-mediated delivery of functionally active miRNA-375-3p mimic regulate epithelial mesenchymal transition (EMT) of colon cancer cells. Life Sci. 2021;269 (15 March):119035.
89. Shams SF, Deyhim M. Platelet-Derived Exosomes as the Nano-Carrier for Mir-150 to Modulate Gene Expression and Cell Cycle in the M07-E Cell Line. J Drug Deliv Sci Technol. 2023; 86(Sep): 104644.
90. Shams SF, Mohammadipour M, Deyhim M. Preparation and characterization of platelet-derived exosomes, as a nanostructure for bio-compound delivery. Nanomed J. 2023;10(2). 131-137.
91. Yao Y, Sun W, Sun Q, Jing B, Liu S, Liu X, et al. Platelet-derived exosomal MicroRNA-25-3p inhibits coronary vascular endothelial cell inflammation through Adam10 via the NF-κB signaling pathway in ApoE−/− mice. Front

Immunol. 2019;10:2205.
92. Tan M, Yan H-B, Li J-N, Li W-K, Fu Y-Y, Chen W, et al. Thrombin stimulated platelet-derived exosomes inhibit platelet-derived growth factor receptor-beta expression in vascular smooth muscle cells. Cell Physiol Biochem. 2016;38(6):2348-2365.
93. Bai X, Zhang H, Li Z, Chen O, He H, Jia X, et al. Platelet-derived extracellular vesicles encapsulate microRNA-34c-5p to ameliorate inflammatory response of coronary artery endothelial cells via PODXL-mediated P38 MAPK signaling pathway. Nutr Metab Cardiovasc Dis. 2022;32(10):2424-2438.
94. Arul S, Peterson T, Behfar A, Kadirvel R, Kallmes D. O-050 Human platelet-derived exosomes as bioactive material for aneurysm treatment with platinum coils: in vitro proof of concept study. BMJ. 2023; 15(Sup1): A39-A40.
95. Hayon Y, Dashevsky O, Shai E, Brill A, Varon D, R Leker R. Platelet microparticles induce angiogenesis and neurogenesis after cerebral ischemia. Curr Neurovasc Res. 2012;9(3):185-192.
96. Wang Z, Zheng Y, Cai H, Yang C, Li S, Lv H, et al. Aβ1-42-containing platelet-derived extracellular vesicle is associated with cognitive decline in Parkinson's disease. Front Aging Neurosci. 2023;15:1170663.
97. Bortot B, Mangogna A, Peacock B, Lees R, Valle F, Brucale M, et al. Platelet Activation in Ovarian Cancer Ascites: Assessment of GPIIb/IIIa and PF4 in Small Extracellular Vesicles by Nano-Flow Cytometry Analysis. Cancers (Basel). 2022;14(17):4100.
98. Vismara M, Zarà M, Negri S, Canino J, Canobbio I, Barbieri SS, et al. Platelet-derived extracellular vesicles regulate cell cycle progression and cell migration in breast cancer cells. Biochim Biophys Acta Mol Cell Res. 2021;1868(1):118886.
99. Jiao Y, Li W, Wang W, Tong X, Xia R, Fan J, et al. Platelet-derived exosomes promote neutrophil extracellular trap formation during septic shock. Crit Care. 2020;24(1):1-18.
100. Lee BK, Kim MH, Lee SY, Son SJ, Hong CH, Jung Y-S. Downregulated Platelet miR-1233-5p in Patients with Alzheimer's Pathologic Change with Mild Cognitive Impairment is Associated with Aβ-Induced Platelet Activation via P-Selectin. J Clin Med. 2020;9(6):1642.
101. Zhu YG, Shi MM, Monsel A, Dai CX, Dong X, Shen H, et al. Nebulized exosomes derived from allogenic adipose tissue mesenchymal stromal cells in patients with severe COVID-19: a pilot study. Stem Cell Res Ther. 2022;13(1):220.
102. Taghiyar L, Jahangir S, Khozaei Ravari M, Shamekhi MA, Eslaminejad MB. Cartilage Repair by Mesenchymal Stem Cell-Derived Exosomes: Preclinical and Clinical Trial Update and Perspectives. *Adv Exp Med Biol*. 2021; 1326:73-93.
103. Rezaie J, Feghhi M, Etemadi T. A review on exosomes application in clinical trials: perspective, questions, and challenges. Cell Commun Signal. 2022; 20(1):145.
104. Johnson J, Law SQK, Shojaee M, et al. First-in-human clinical trial of allogeneic, platelet-derived extracellular vesicles as a potential therapeutic for delayed wound healing. J Extracell Vesicles. 2023;12(7):e12332.
105. Skuratovskaia D, Vulf M, Khaziakhmatova O, Malashchenko V, Komar A, Shunkin E, et al. Exosome limitations in the treatment of inflammatory diseases. Curr Pharm Des. 2021;27(28):3105-3121.