

REVIEW PAPER

## The role of platelet derived exosomes in regenerative medicine

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### 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

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### 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  $\mu\text{m}$ . 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- $\beta$  (TGF- $\beta$ ), and vascular endothelial growth factor/vascular permeability factor (VEGF/VPF). Platelets also make other bioactive compounds such as extracellular vesicles [4,

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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, Nilforoushadeh 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  $\alpha$ -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  $\alpha$ -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  $\alpha$ -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- $\beta$ ) 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- $\beta$  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 ubiquitination 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  $\alpha$ 1) 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- $\beta$ , and MMP-3, and also decrease the expression of miRNA-203 (targeting the proliferative and migratory factors at the site of injury) and  $\alpha$ SMA 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 myogenesis, 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]. In vitro 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

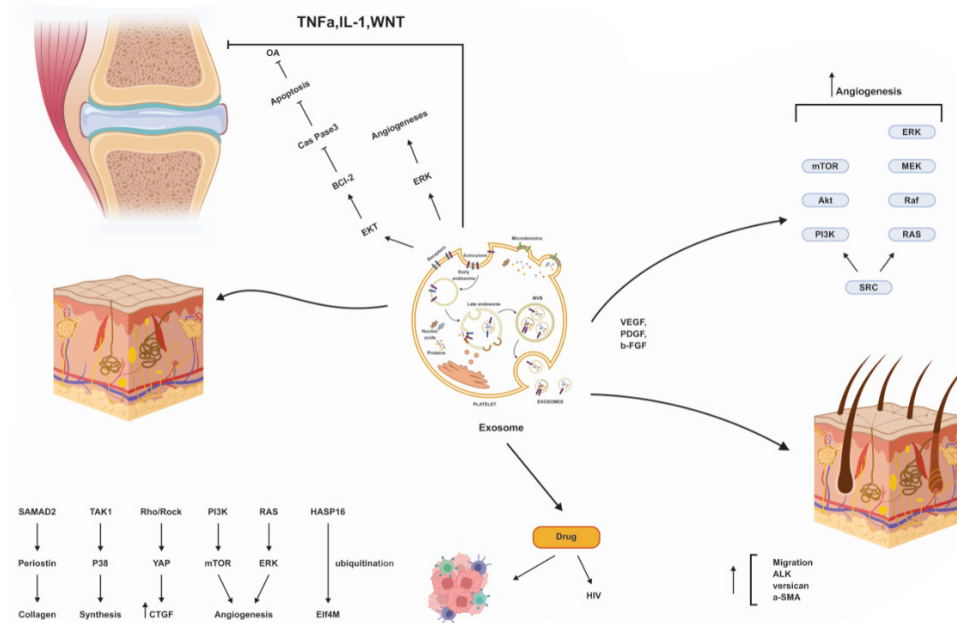


Fig.1. Role of platelet exosomes in regenerative medicine. Platelet growth factors present in exosomes cause hair repair by increasing the expression of ALP, versican and  $\alpha$ -SMA. By stimulating the ERK and PI3K signaling pathway, they increase angiogenesis. By inhibiting inflammatory cytokines and wnt/ $\beta$ -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  $\beta$ -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-myc 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<sup>-/-</sup> 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- $\kappa$ B 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 (PDGFR $\beta$ ), 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  $\beta$  1-42 is associated with dementia. It has been shown that platelet exosomes in patients with Parkinson's disease and dementia are rich in A $\beta$  1-42. Accordingly, the use of platelet exosomes and their A $\beta$  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  $\beta$  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 amyloid $\beta$

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.

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## DATA AVAILABILITY

All the data have been included 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.

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