IJHOSCR

Review Article

International Journal of Hematology-Oncology and Stem Cell Research

A Mini-Review on Fibroblast-Derived Exosomes as Wound Healing Stimulators

Alireza Sadeghi Moghaddam Bijari[1], Mahdi Alijanianzadeh[1], Hoda Keshmiri Neghab[2], Mohammad Hasan Soheilifar[2]

[1]Department of Cell & Molecular Biology, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran
[2]Department of Medical Laser, Medical Laser Research Center, Yara Institute, ACECR, Tehran, Iran

Corresponding Author: Hoda Keshmiri Neghab, Department of Medical Laser, Medical Laser Research Center, Yara Institute, ACECR, Tehran, Iran
E-mail: hodakeshmiri@ut.ac.ir

Received: 12, Aug, 2024
Accepted: 03, Mar, 2025

ABSTRACT

The skin is a vital organ that plays a crucial role in healing disruptions and abnormalities. Cutaneous wound healing faces some obstacles in certain abnormalities, including in diabetic patients. Various therapeutic approaches have been explored to enhance healing and restore skin integrity.

In recent years, exosomes have been introduced as a new cell-free therapy for wound healing. They are defined as naturally secreted nanovesicles released from most cell types into the extracellular space that can impact many targeted cells. In contrast to previous methods, exosomes have a longer half-life in target tissue and exert a more lasting effect. They also have fewer side effects thanks to their natural biological source.

Exosomes derived from mesenchymal stem cells (MSCs) have been widely studied for their therapeutic potential, but those from other cell types, such as fibroblasts, remain less explored. This review aims to comprehensively evaluate existing research on the wound-healing effects of fibroblast-derived exosomes (FB-EXOs), highlighting their potential as a novel treatment strategy.

Keywords: Exosomes; Fibroblasts; Wound healing; Stem cell research

INTRODUCTION

The skin is the largest organ in the human body that accounts for around 16% of a person's total body weight. It plays a crucial role in body maintenance, including maintenance of body fluids and electrolytes and protection against pathogens and physical strokes. Since it often suffers from various damages such as wounds, its structure needs to be repaired after injuries. Despite proper wound healing under normal conditions, patients face improper skin healing in chronic and acute wounds. Moreover, wound healing culminates in scar formation even in lots of normal cases[1-3].

Proper wound healing involves multiple factors and regulates the triggering of complex signaling pathways. The absence of these participants leads to a disrupted wound-healing process. For example, diabetes mellitus (DM) is a metabolic disorder caused by a lack of insulin production characterized by irregular blood glucose concentration[4]. In diabetic patients, overactivity of aldose reductase (ALR2) leads to high levels of sorbitol and fructose and their aggregation, which results in reduced nicotinamide adenine dinucleotide (NADH). NADH is critical to reactive oxygen species (ROS) detoxification and nitric oxide (NO) synthesis. Together, these lead to neuropathy, which in turn, causes anatomical foot deformities and, consequently, a skin disruption known as diabetic foot ulcer (DFU)[5,6]. DFU affects approximately 15% of patients with DM, and in many cases, leads to their hospitalization or even death. DFU also has several side effects, including infection and gangrene, which increase the risk of amputation[7,8].

To solve these problems, many efforts have been made, and several advantageous methods have been proposed, including bioengineered skin, laser therapy, electrostimulation, business growth factors, negative pressure wound therapy, and recently proposed stem cell therapy [2,3] .Given that stem cells impress mainly with their secretome, recent studies have suggested that stem cell-secreted materials could be applied, especially when they are packaged in the form of bilayer nanovesicles in the size range of 30-200 nm, the Exosomes, in charge of carrying signalosomes and transferring biomolecules to specific receptors of target cells. These extracellular vesicles (EVs) are composed of lipids, proteins, and carbohydrates and carry various cargoes, including proteins, nucleic acids, and lipids [9]. They are released from different types of cells such as keratinocytes, adipocytes, MSCs, and fibroblasts [1,10]. Every cell type releases these vesicles with various sizes and cargos that impress target tissue cells, depending on their role and duty. While the particles derived from certain cell types, especially MSC-derived exosomes (MSC-EXOs), have been well studied, and their positive effects on wound and skin healing have been clearly demonstrated, some others like fibroblast-derived exosomes (FB-EXOs) are still not well known, and less is known about their effects on skin and wound healing. Nevertheless, this review introduced fibroblasts and FB-EXOs and assessed the effects and functions of FB-EXOs on skin development, especially wound healing. Its content has been explained, and innovations have been implied in its application.

Wound healing process

In general, wound healing is divided into 4 phases: homeostasis and inflammation, cell proliferation, and matrix remodeling. Just after injury, a fibrin network forms to make a platform for the agglutination and accumulation of cells like platelets. After agglutination, platelets and other immune cells initially induce an inflammatory response in the wound area and then promote fibroblast migration and proliferation in this zone. In the next step, where fibroblasts play a critical role, cell proliferation and extracellular matrix (ECM) secretion are essential for new tissue formation. Finally, ECM secretion and processing will continue until the maturation of early secreted frail ECM [11,12.]

Cell therapy

In recent years, some cells have been directly applied to the wound area as an effective wound-healing method called “cell therapy”. Various cell types like stem cells and blood cells have been investigated and demonstrated some positive effects[13,14]. Stem cells play an important role in wound healing and skin regeneration because of their strong self-renewal and differentiation ability in the process of wound treatment, using stem cells can close the wound early and reduce scar formation[15].

Direct application of stem cells faces several shortcomings and obstacles, including immune rejection, low viability of transplanted cells, and risk of tumorigenicity. Besides, the effects of cells on cutaneous damage can be mostly due to their secreted EVs rather than their stemness properties. Thus, it is recommended to apply pure EVs to the wound area .EV overcomes the limitations of stem cells[16–18.]

Exosomes

Exosomes are nano vesicles that released from various cell types including such as fibroblasts [16,19]. These EVs are a complex package of various proteins (e.g., receptors, transcription factors, enzymes, etc.), lipids, and nucleic acids (e.g., mRNA, miRNA, lncRNA, and DNA)[20,21]. Their biogenesis begins with the invagination of the cell membrane to form an early endosome. Early endosomes in cytoplasm mostly mature and attract multiple cargos to form multivesicular bodies (MVBs). Most MVBs are directed to lysosomes to recycle, while some mature into late endosomes and, finally, are released to EVs such as exosomes[22]. Compared to direct cell therapy, these agents less stimulate the immune system, are more stable in body fluids and tissues, and have high specificity and potency in intended tissue targeting, due to their unique structure[9]. All these advantages make exosomes promising alternatives to traditional methods.

Role of fibroblasts in skin and skin repair

Fibroblasts are differentiated from MSCs and exist in most organs of the body. They are particularly the producers as they secrete components like collagens, glycosaminoglycans (GAGs), elastic fibers, and glycoproteins, and contribute to connective tissue formation. Following skin damage, these cells proliferate and migrate to the wound area and participate in tissue repair [23–25]. In cases of damage, some fibroblasts can also differentiate into most abundant cells in the dermal layer and have a spindle-shaped monolayer morphology with a diameter of 100-200 µm. They also act as main ECM myofibroblasts. This change increases their ECM secretion potential [26].

In addition to ECM production, fibroblasts can also regulate multiple processes, including Wnt signaling pathways, which are critical to promoting skin differentiation. They perform this task by secreting various components such as growth factors (TGFb-1, FGF, KGF, etc.)[19,24,27].

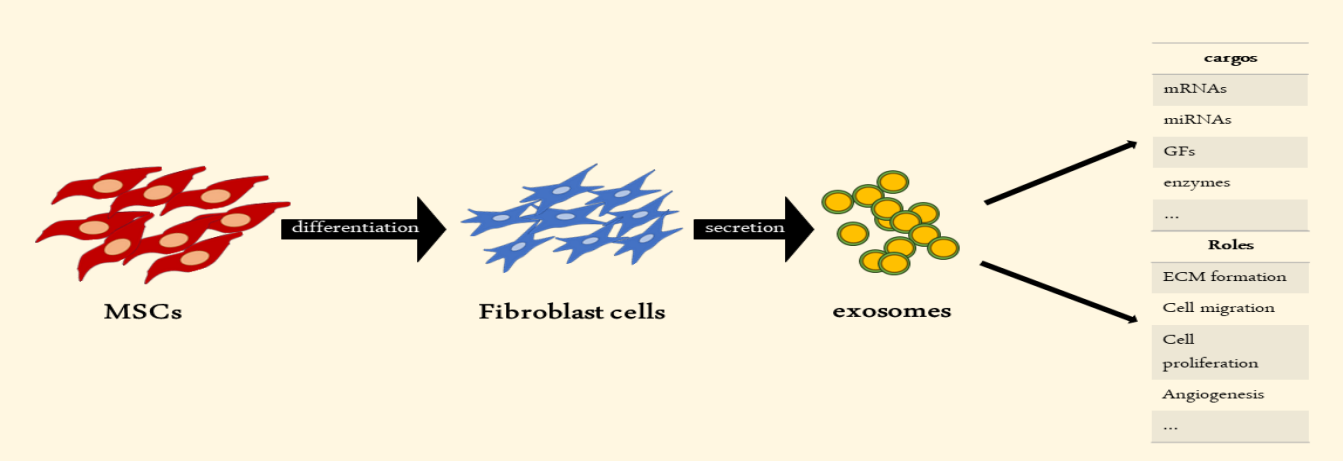

Figure 1. Fibroblast cells differentiate from MSCs and secret exosomes that carry various types of cargos and effect on wound healing

Advantages of FB-EXOs

Exosomes must be easily isolated from various sources with a high exosome production rate to be effectively used. Despite their functions, cultivation of MSCs takes a lot of time and effort, and they are isolated from certain tissues using an invasive method. Moreover, the risk of red blood cell (RBC) contamination inevitably reduces cell viability and neovascularization potential [28]. Hence, a new easier, faster, and safer source of exosome isolation is required. In recent studies, fibroblasts have been recommended due to their simple isolation from skin using less invasive methods and their faster growth compared to MSCs. In addition, fibroblasts are more specialized cells associated with skin health and wound healing processes [28,29]. As an example of this specificity, a comparative study showed that fibroblast exosomes induced more than 2-fold more collagen 3 gene expression than fibroblast exosomes over a 3-day period. Overall, as a suitable alternative to MSC-EXOs, FB-EXOs are known to promote wound healing [28,30].

Therapeutic applications of FB-EXOs on wound healing

Several studies have been carried out on the positive therapeutic effects of FB-EXOs on wound healing promotion. An *in vivo* study was conducted on 48 male Wistar rats, where wounds were made on their back skin and treated with exosomes in high-dose (HDE) and low-dose (LDE) groups. Commercial ointment was used as a positive control group [27].

Based on the results, exosome treatments have more significant effects on wounds compared to commercial ointments. HDE especially has higher levels of epithelialization and fibrosis. It also had higher crust formation in the early days but zero crust formation on day 12. Clearly, while all three treatment groups have positive effects, exosome treatments appear to be more effective [27].

Table 1. Summary of recent years' reports about fibroblast derived exosomes effects on skin health

Source	Research method	Research target	Year	Reference
Mouse wound-edge fibroblasts	In vitro/in vivo	Aged cutaneous wound	2022	(31)
Human dermal fibroblasts	In vitro	Skin development	2022	(32)
Human dermal fibroblasts	In vitro/in vivo	Ischemic wound healing	2021	(16)
CHDFs and LWDFs	In vitro/in vivo	Skin development	2022	(33)
Human dermal fibroblasts	In vitro/in vivo	Aged cutaneous wound	2020	(34)
Human dermal fibroblasts	In vitro/in vivo	Skin aging	2019	(25)
NF1 human dermal fibroblast	In vitro	Neurofibromatosis type 1	2023	(35)
Human dermal fibroblast	In vitro	Systemic sclerosis	2022	(36)
Mouse fibroblast cell line L929	In vivo/ in vitro	Wound healing	2020	(28)
Mice dermal fibroblast	In vivo/in vitro	Diabetic Wound Healing	2022	(37)
Rat dermal fibroblast	In vivo/in vitro	Diabetic Wound Healing	2021	(6)
Human Fetal Skin Fibroblast	In vivo/in vitro	Wound healing	2023	(27)
Hypertrophic Scar Fibroblast/ Normal fibroblast	In vitro	Skin development	2023	(38)
Human dermal fibroblast	In vitro	Wound healing	2023	(39)
Human skin fibroblast	In vivo/in vitro	Wound healing	2024	(40)

Another study also approved this claim that FB-EXOs have beneficial effects on wound healing, along with exosomes derived from epidermal stem cells (ESC-EXOs). After applying FB-EXOs, epidermal thickness and macrophage transformation increased, while the width of the wound diminished compared to the PBS control group .However, ESC-EXOs also exert effects [37].

In another study, the L929 mouse fibroblast cell line was selected as a source of exosome isolation, which was demonstrated to affect both fibroblast and endothelial cells. Interestingly, after the internalization of L929-EXOs into fibroblasts, increasing migration and proliferation were observed in a dose-dependent manner. As a result of the wound healing/migration assay, fibroblasts showed increased migration rates at both 18 and 36 hours. L929-EXOs upregulate the expression of three genes associated with scarless wound healing, including MMP1, MMP3, and COL3A1. However, this effect was shown to be higher in an optimal concentration of exosomes [28].

In addition to fibroblasts, FB-EXOs also promote the migration and tube formation ability of endothelial cells (ECs). ECs are active players in the formation of new blood vessels (angiogenesis), which is a critical step in wound healing. Expression of vascular endothelial growth factor (VEGF) as an essential factor for angiogenesis was quantified.

It slightly increased in the initial days but significantly increased in the following days, as compared to other groups [28,41]. CD31, the marker protein of ECs, was measured in one study and revealed that FB-EXOs increase its expression 3fold more than normal control [6].

Another effect of these exosomes is hair growth initiation. Proper wound healing involves restoring lost hair follicles and growing new hair. β-catenin is a hair growth promoter, which was highly expressed in the L929-EXOs group at day 8, whereas no β-catenin expression was observed in fibrin glues (FG) and control treatment groups [28].

Wound healing in diabetes

Diabetes impairs the wound healing process due to some obstacles. For example, high oxidative stress in diabetic environment, damage DNA, proteins, and lipids within cells, ultimately resulting in cell death and consequent tissue malfunction. High level of glucose causes a disruption in lipid metabolism that may result in aberrant immune cells and signal transmission. Angiogenesis also disrupts in result of

high glucose condition. So, diabetic wounds can't heal properly without intervention [42].

Recent studies have suggested that FB-EXOs can fight diabetic foot ulceration through neovascularization. It is assumed that FB-EXOs activate the Akt/β-catenin pathway, a fundamental pathway to wound healing advancement. High glucose levels can diminish the proliferation, migration, and tube formation ability of human dermal microvascular endothelial cells (HDMECs) and human umbilical vein endothelial cells (HUVECs). However, FB-EXOs are potent enough to restore these abilities. In a study on diabetic rats, it was observed that wounds treated with FB-EXOs had almost closed after 15 days. Moreover, in collagen deposition analyses, FB-EXO treatment made large amounts of collagen fibers in a well-designed form compared to the PBS treatment. Blood vessels in the DF-EXO treatment group formed in larger numbers and bigger sizes [6].

Wound healing in aged skin

Besides diabetes, aging is also an important factor associated with delayed and impaired wound healing. Thus, people over 65 years of age show significantly delayed wound healing[43]. Reduced keratinocyte, fibroblast, mast cell, and macrophage proliferation and ECM production in the skin gradually occur with aging. In addition, the lack of skin elasticity is attributed to the degraded elastin morphology [10].

In wound healing, new tissue formation requires ECM deposition. For this purpose, fibroblasts should differentiate into myofibroblasts, which especially secret ECM to resilient tissues. In the general process, fibroblasts correctly differentiate into myofibroblasts but skin senescence leads to defective fibroblast-to-myofibroblast transition (FMT) and dysfunctional ECM deposition [31].

Thanks to their regenerative abilities, FB-EXOs are applied to aged skin wounds to ameliorate senescence and cutaneous wounds. According to an *in vivo* assessment of wound closure in mice, divided into two treatment groups (young skin and aged skin derived exosomes), the aged group exhibited poorer wound closure ability than the young group. The aged group had lower amounts of collagen in the wound area than the young group. Besides, an increase in matrix metalloproteinases (MMPs) in the aged group resulted in ECM breakdown. Subcutaneous injection of FB-EXOs in the aged skin of mice was shown to boost wound closure. In the young group, α-SMA (i.e., the most common myofibroblast marker) showed higher expression levels. Interestingly, exosomes secreted by young fibroblasts were indicated to be more effective than those secreted by old fibroblasts. They can elevate collagen I deposition. Higher α-SMA expression levels or wound closure rates were observed in the young exosome-treated group [31].

ECM proteins within tissues are sometimes degraded for various physiological or pathogenic purposes. With aging, ECM protein production decreases, and ECM degradation occurs more often in aged cases than in young cases. MMPs are enzymes especially responsible for ECM degradation. To prevent ECM degradation, fibroblasts secret tissue inhibitors of MMPs (TIMPs) via their exosomes. The TIMP family contains four members: TIMP-1, TIMP-2, TIMP-3, and TIMP-4. Since FB-EXOs are enriched with TIMPs, particularly TIMP-1, they can reduce the MMP activity to normal levels. This regulation is critical for maintaining ECM. Although the reproductive potential of the skin diminishes as a result of senescence, there is promising evidence that EXOs secreted by young fibroblasts can refine this ability, and aged skin wounds can heal almost as well as young skin wounds [25,44].

Effects of FB-EXOs on other skin disorders

While skin wounds are crucial, skin injuries are not limited to wounds. Various diseases can disrupt the skin structure and function, including eczema, acne, dry skin (aka xeroderma), atopic dermatitis (AD), etc[45].

For example, the effect of FB-EXOs on AD was evaluated. The skin permeability barrier helps retain water and electrolytes in the body and prevent their loss. This skin function is disrupted by some diseases such as AD [46] . A study investigated how FB-EXOs could help repair the skin permeability barrier. The main marker of the skin permeability barrier is the downregulation of IVL, LOR, FLG, and HAS1 expression. It was observed that FB-EXOs restored

the expression potential of IVL, LOR, FLG, and HAS1 keratinocytes that were treated with 1-chloro-2,4-dinitrobenzene (DNCB) to reduce the expression of this marker. FB-EXOs have shown significant beneficial effects on AD, especially at certain concentrations [29].

Mechanisms of Action

Wnt/ β-catenin signaling pathway

The wingless-related integration site (Wnt) signaling pathway is an essential regulator of many biological processes. The Wnt pathway, especially the β-catenin-depended pathway, affects the expression of homeostasis- and fibrosis-related genes. In the absence of a Wnt signal, β-catenin is suppressed and degraded by a complex of proteins. However, in the presence of the Wnt signal, β-catenin translocates from the cytosol to the nucleus and activates the transcription of target genes by binding to TCF/LEF transcription factors. The Wnt/β-catenin pathway plays a key role in all phases of the wound healing process. Owing to their lipophilic structure, Wnt proteins should be carried by exosomes. Fibroblasts can trigger fibrogenesis and are also involved in tissue homeostasis via the secretion of Wnt-containing exosomes. Accordingly, these exosomes can participate in wound healing processes [27,47].

FB-EXO miRNAs

MiRNA 125b (miR-125b)

MicroRNAs are short noncoding RNAs that act as regulators of protein mRNAs [48]. Sixty-six miRNAs have been found to alter expression during skin disruption. MiR-125b is one of the most important regulators that is expressed differentially in the wound area[49]. Assessments have revealed that young FB-EXOs (e.g., miR-125b) are more effective than exosomes that inhibited their miR-125b expression in ECM deposition and during the wound healing process [33].

TGF-β appears to be an underlying wound-healing promoter, which can upregulate most target proteins, including SMAD2, RAS, MMK3, RHOA, ERK1, TAK1, PERIOSTIN, and P38. These are correlated with fibroblast wound healing activities and epithelial transdifferentiation. Mir-125b mainly regulates the expression of TGF-β signaling factors. Interestingly, exosomes neutralized by the TGF-β antibody revealed suppressed fibroblast activity, keratinocyte proliferation and migration, ECM deposition, and tube formation of HUVECs [16.]

Sirtuin-7 (Sirt7) is an NAD^+-dependent protein deacetylase that participates in diverse crucial biological processes including aging [50]. Sirt7, a direct target of miR-125b, reduces the cell migration and FTM of fibroblasts. According to analyses, treatment with young FB-EXOs via miR-125b reduces the expression of sirt7 more than the aged and control groups. FB-EXOs via miR-125b can overcome impaired wound healing in aged skins[31].

MiR-125b also impresses angiogenesis in injured skins via regulation of VEGF. VEGF is an important growth factor that stimulates angiogenesis in the early stages of wound healing. According to observations, it was downregulated by miR-125b three days after injury. After day 3, it was gradually upregulated, leading to reduced VEGF. In the initial days, damaged tissues required promoting angiogenesis, whereas angiogenesis needed to be regulated in the later stages of wound healing. Hence, miR-125b plays a key role in the regulation of angiogenesis [49].

MiR-218

MiR-218 is another FB-EXO cargo associated with collagen I deposition and fibroblast proliferation and migration. This hypothesis has been proven by inhibition of miR-218 by its inhibitors. Proliferation, migration, and collagen I release upregulated by miR-218 notably decreased after inhibition. *In vivo* assessments indicated a gradual increase in the thickness of mouse skin[33].

TGF-β-induced factor homeobox 2 (TGIF2) has been detected as a target of miR-218, which could inhibit TGF-β1, an underlying wound healing promotion factor. MiR-218 has a complementary sequence between the 3'UTRs of the TGIF2 gene, thus inhibiting its transcription. While TGIF2 diminishes TGF-β1 transcription in the absence of miR-218, TGF-β1 upregulates and modulates skin development in its presence. Unlike TGIF2, many proteins upregulate the impression of FB-EXOs via miR-218. ITGBL1 is one of the most upregulated proteins, which can activate

multiple signaling pathways, including TGF-β1/SMAD2/3, when it increases[33]. To improve the hypothesis that reduced miR-218 leads to alleviated fibrosis, fibroblast activity in dermal and gingival fibroblasts was evaluated.

Gingival fibroblasts do not contribute to scar formation because they release relatively less ECM than dermal fibroblasts. It was observed that miR-218 decreased in gingival fibroblasts, leading to diminished TGF-β1. The absence of TGF-β1 can be attributed to less deposition of ECM in tissues [51].

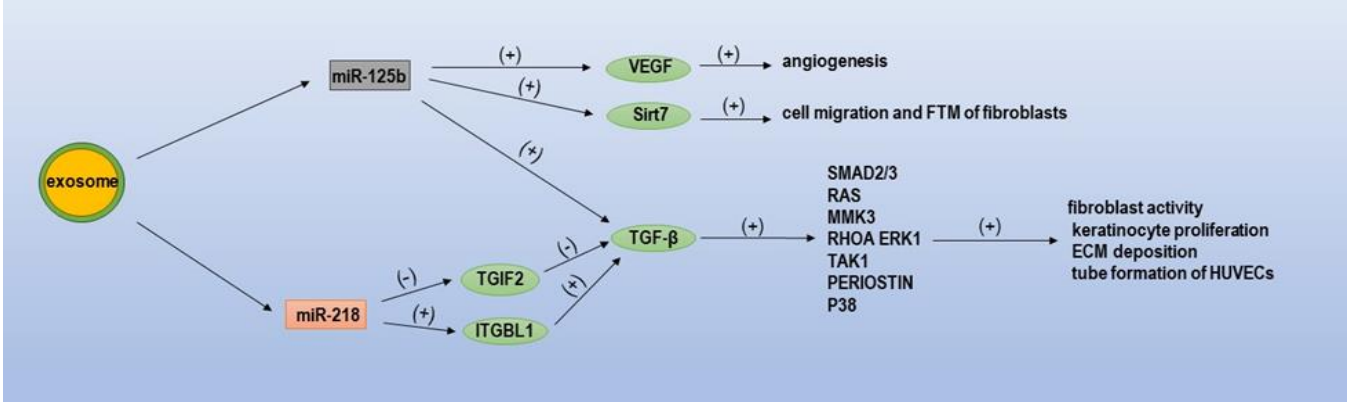

Figure 2. FB-EXOs carry some important miRNAs that regulate the expression of signaling proteins to healing the wound

Innovative methods for improving the effectiveness of FB-EXOs on wound healing

While FB-EXOs show remarkable efficacy merely in skin growth, it would be interesting to know if their effect is maximized in combination with other components or in an innovative way of application. This section, briefly introduces some innovative methods.

Fibrin glue (FG)

Proper wound healing necessitates gradual and sustained release of exosomes in the wound area. Meanwhile, exosomes are highly internalized by target cells and have a very short half-life in extracellular environments [16]. A solution is the use of a hydrogel carrier to make an environment for EVs. Among the different types of hydrogels, FGs (containing fibrin and thrombin) are commonly used in clinical practices and can accelerate wound closure. Though FGs can be used alone, they are mostly applied in the early days. Also, they can be enriched with biological additives. Based on observations, EXOs in combination with FGs are more effective than either alone [28,52,53].

It has been suggested that FB-EXOs can be combined with TISSEEL, a commercially available fibrin sealant, and applied to wounds. This combined treatment has been shown to have more benefits than each one individually. The application of TISSEEL-EXOs on rabbit ischemic wounds did not leave any scars on the skin, while the wound area was still observed in the TISSEEL, exosome, and normal control groups. In addition to proliferation and migration, TISSEEL-EXOs promote hair growth earlier than others. TISSEEL-EXO treatment showed higher skin hydration and fat levels (other key features to assess skin health) than other treatment groups [16].

FB-EXOs induce the release of type I and III collagen. However, in the case of TISSEEL-EXO treatment, the Col III/Col I ratio is higher than in other gels. Interestingly, this phenomenon is associated with scar formation since higher Col III levels lead to less scar formation [16].

Three-dimensional culture

In recent years, it has been proposed that cells be cultured in 3D to simulate the native environment. Since 3D cell culture makes the conditions more

similar to tissue, cell activation under these conditions can be considered similar to the *in vivo* environment with more certainty [54].

In a study, the effect of 3D culture of fibroblasts on exosome content was demonstrated by *in vitro* and *in vivo* studies on senescent cells. 3D FB-EXOs significantly increased the expression of TGF-β1 and TIMP1 proteins compared to 2D FB-EXOs. Additionally, 3D FB-EXOs have higher levels of hsa-miR-133a-3p, hsa-miR-223-3p, hsa-5011-5p, hsa-miR-325, has-miR-199b-5p, and hsa-miR-34a-5p and lower levels of hsa-miR-196a-5p and hsa-miR-744-5p, compared to 2D HDF-XOs. Each of these changes in exosome content leads to a higher potential for regulating different wound-healing signaling pathways. For example, miR-223 upregulates IL-10 and downregulates TNF-α [25,55,56].

In another study, the difference between 2D and 3D fibroblast cultures and their exosomal properties were evaluated. Regarding RNA content, there are 3730 differentially regulated though highly similar genes, including 2469 upregulated genes and 1261 downregulated genes in 3D-cultured fibroblasts. Consequently, 217 proteins were modulated in the 3D culture, including 105 upregulated proteins and 112 downregulated proteins, compared to the 2D culture [32].

To demonstrate the advantage of 3D FB-EXOs, fibroblast monolayer cultures were treated with 3D and 2D FB-EXOs. 3D EXOs were clearly superior in inducing cell migration. Thus, it can be concluded that the culture of fibroblasts in spheroid form can enhance the therapeutic potential of their exosomes compared to the conventional monolayer cell culture method [32].

FB-EXOs derived from thicker skins and wound healing

Research on fibroblast behaviors has demonstrated a direct relationship between skin thickness and fibroblast activity, so the thicker the skin, the more and more effective exosomes are secreted. As an example, fibroblasts from animals with thicker skin like Chenghua pig (CHs) have been found to release exosomes that affect the proliferation and migration of target cells more strongly than others with thinner skins like large white pig (LWs)[33].

systemic sclerosis (SSC) is an autoimmune disease that affects the skin and internal organs. SSC patients have thicker skins than normal people[57,58]. In another study, it has been reported that the number of exosomes in SSC patients increases compared to normal cases. SSC-FB-EXOs upregulate all of inflammatory factors including IL-1α, TNF, and lymphotoxin (TNF-β) and Regulators of fibrosis including IL-6, IL-10, IL-12p40, IL-22, VEGF, and CCL2more than normal fibroblasts, and include. However, the risk of scar formation is controversial and needs to be further investigated [36].

Needle-free injection

When it comes to skin treatments, conventional methods such as local drug injection play a major role. Jet injection (i.e., a needle-free injection device that forces a stream of liquid into the skin and subcutaneous tissue) has been introduced as an alternative to traditional syringe-based injection, which has been practiced predominantly until today. Unlike traditional injection, jet injection does not cause pain or bruising in the skin. Fortunately, this method can also be used for exosome injection, as it has proven successful in injecting FB-EXOs to improve the skin [25,59].

Mix therapy

The efficacy of FB-EXOs can be enhanced in synergism with other components. A recent study has tried to reveal the effect of FB-EXOs containing the Matrine, a well-studied component found in Sophora flavescens. Matrine and FB-EXO both have positive effects on wound healing. However, in mix application, wound healing rate has an increase in in *vitro/in vivo* tests[40].

CONCLUSION

Based on the data, fibroblasts are important cells in the regulation and progression of skin repair, especially in wound healing. Exosomes secreted from fibroblasts have been shown to be promising in promoting skin repair. They can increase cell proliferation and migration rates in many types of cells, including ECs and fibroblasts. FB-EXOs increase the ECM production rate both directly and indirectly, induce angiogenesis in skin tissue, and stimulate hair

growth. They can even affect macrophages, thereby interfering with immune regulation.

The function of FB-EXOs can be altered in some cases of abnormality that may be beneficial or dangerous. These alterations may lead to changes in the wound healing process and scarring.

It has also been proven that skin thickness is related to fibroblast activity. Exosomes isolated from fibroblasts of thicker skins are more active and effective than others. They can regulate some signaling pathways such as Wnt through certain components like miRNAs.

In addition to the effectiveness of FB-EXOs individually, many efforts have been made to improve their effects using innovative methods of application. Despite these advantages, FB-EXOs obviously face some limitations and challenges. For example, their effects are related to the age of the extraction source as it is diminished by the old skin-isolated exosomes. Therefore, old sources can diminish the effect. Accordingly, the thinner the skin, the weaker the effect becomes.

Since 2D cell culture may prevent exosomes from demonstrating their maximum potential, 3D cell culture methods are recommended.

Ultimately, given their specificity and focused effect on ECM formation, it is preferable to use FB-EXOs in combination with other types of exosomes as part of combination therapy to address all dimensions of the wound healing process.

Collectively, FB-EXOs can heal skin wounds caused by health disorders such as diabetes and aging and improve other skin disorders such as AD. However, further investigation is required to better understand the effect of FB-EXOs on various skin defects and reveal the mechanisms of skin repair. Furthermore, FB-EXOs should be compared with all other sources to find the best source of exosomes.

CONFLICTS OF INTEREST

There are no conflicts of interest.

REFERENCES

1. Liu Y, Wang H, Wang J. Exosomes as a novel pathway for regulating development and diseases of the skin. Biomed Rep. 2018;8(3):207–14.

2. Rani S, Ritter T. The exosome-A naturally secreted nanoparticle and its application to wound healing. Adv Mater. 2016;28(27):5542–52.

3. Broughton II G, Janis JE, Attinger CE. Wound healing: an overview. Plast Reconstr Surg. 2006; 117(7Suppl):1e-S-32e-S.

4. Deshmukh CD, Jain A, Nahata B. Diabetes mellitus: a review. Int J Pure App Biosci. 2015;3(3):224–30.

5. Li X, Xie X, Lian W, et al. Exosomes from adipose-derived stem cells overexpressing Nrf2 accelerate cutaneous wound healing by promoting vascularization in a diabetic foot ulcer rat model. Exp Mol Med. 2018;50(4):1–14.

6. Han X, Wu P, Li L, et al. Exosomes derived from autologous dermal fibroblasts promote diabetic cutaneous wound healing through the Akt/β-catenin pathway. Cell Cycle. 2021;20(5–6):616–29.

7. Snyder RJ, Hanft JR. Diabetic foot ulcers--effects on QOL, costs, and mortality and the role of standard wound care and advanced-care therapies. Ostomy Wound Manage. 2009;55(11):28–38.

8. Yazdanpanah L, Nasiri M, Adarvishi S. Literature review on the management of diabetic foot ulcer. World J Diabetes. 2015;6(1):37-53.

9. Chavda VP, Pandya A, Kumar L, et al. Exosome nanovesicles: A potential carrier for therapeutic delivery. Nano Today. 2023;49:101771.

10. Hajialiasgary Najafabadi A, Soheilifar MH, Masoudi-Khoram N. Exosomes in skin photoaging: biological functions and therapeutic opportunity. Cell Commun Signal. 2024;22(1):32.

11. Gonzalez AC de O, Costa TF, Andrade Z de A, et al. Wound healing-A literature review. An Bras Dermatol. 2016;91(5):614–20.

12. Enoch S, Leaper DJ. Basic science of wound healing. Surgery (Oxford). 2008;26(2):31–37.

13. Mester A, Opincariu D, Benedek I, et al. Stem cell therapy in wound healing. J Interdiscip Med. 2017;2(s4):20–4.

14. Oneto P, Etulain J. PRP in wound healing applications. Platelets. 2021;32(2):189–99.

15. Lv H, Liu H, Sun T, et al. Exosome derived from stem cell: A promising therapeutics for wound healing. Front Pharmacol. 2022;13:957771.

16. 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.

17. Wong VW, Sorkin M, Gurtner GC. Enabling stem cell therapies for tissue repair: current and future challenges. Biotechnol Adv. 2013;31(5):744–51.

18. Duscher D, Barrera J, Wong VW, et al. Stem cells in wound healing: The future of regenerative medicine? A mini-review. Gerontology. 2016;62(2):216–25.

19. desJardins-Park HE, Foster DS, Longaker MT. Fibroblasts and wound healing: an update. Regen Med. 2018;13(5):491-495.
20. Patil AA, Rhee WJ. Exosomes: biogenesis, composition, functions, and their role in pre-metastatic niche formation. Biotechnology and Bioprocess Engineering. 2019;24:689–701.
21. Li X, Corbett AL, Taatizadeh E, et al. Challenges and opportunities in exosome research—Perspectives from biology, engineering, and cancer therapy. APL Bioeng. 2019;3(1):011503.
22. Yue B, Yang H, Wang J, et al. Exosome biogenesis, secretion and function of exosomal miRNAs in skeletal muscle myogenesis. Cell Prolif. 2020;53(7):e12857.
23. Nilforoushzadeh MA, Ashtiani HRA, Jaffary F, et al. Dermal fibroblast cells: biology and function in skin regeneration. J Skin Stem Cell. 2017; 4(2):e69080.
24. Haniffa MA, Collin MP, Buckley CD, et al. Mesenchymal stem cells: the fibroblasts' new clothes? Haematologica. 2009;94(2):258-63.
25. Hu S, Li Z, Cores J, et al. Needle-free injection of exosomes derived from human dermal fibroblast spheroids ameliorates skin photoaging. ACS Nano. 2019;13(10):11273–82.
26. D'Urso M, Kurniawan NA. Mechanical and Physical Regulation of Fibroblast–Myofibroblast Transition: From Cellular Mechanoresponse to Tissue Pathology. Front Bioeng Biotechnol. 2020:8:609653.
27. Ahmadpour F, Rasouli HR, Talebi S, et al. Effects of exosomes derived from fibroblast cells on skin wound healing in Wistar rats. Burns. 2023; 49(6):1372-1381.
28. Oh EJ, Gangadaran P, Rajendran RL, et al. Extracellular vesicles derived from fibroblasts promote wound healing by optimizing fibroblast and endothelial cellular functions. Stem Cells. 2021;39(3):266–79.
29. Yoo K, Thapa N, Lee J, et al. Dermal fibroblast cell-derived exosomes for atopic dermatitis: in-vitro test. Skin Res Technol. 2023;29(7):e13382.
30. Zhang J, Guan J, Niu X, et al. Exosomes released from human induced pluripotent stem cells-derived MSCs facilitate cutaneous wound healing by promoting collagen synthesis and angiogenesis. J Transl Med. 2015:13:49.
31. Xia W, Li M, Jiang X, et al. Young fibroblast-derived exosomal microRNA-125b transfers beneficial effects on aged cutaneous wound healing. J Nanobiotechnology. 2022;20(1):144.
32. Clément V, Roy V, Paré B, et al. Tridimensional cell culture of dermal fibroblasts promotes exosome-mediated secretion of extracellular matrix proteins. Sci Rep. 2022;12(1):19786.
33. Zou Q, Zhang M, Yuan R, et al. Small extracellular vesicles derived from dermal fibroblasts promote fibroblast activity and skin development through carrying miR-218 and ITGBL1. J Nanobiotechnology. 2022;20(1):296.
34. Fafián-Labora JA, Rodríguez-Navarro JA, O'Loghlen A. Small extracellular vesicles have GST activity and ameliorate senescence-related tissue damage. Cell Metab. 2020;32(1):71–86.e5.
35. Roy V, Paquet A, Touzel-Deschênes L, et al. Heterozygous NF1 dermal fibroblasts modulate exosomal content to promote angiogenesis in a tissue-engineered skin model of neurofibromatosis type-1. J Neurochem. 2023;167(4):556–570.
36. Bhandari R, Yang H, Kosarek NN, et al. Human dermal fibroblast-derived exosomes induce macrophage activation in systemic sclerosis. Rheumatology (Oxford). 2023;62(SI):SI114-SI124.
37. Wang P, Theocharidis G, Vlachos IS, et al. Exosomes derived from epidermal stem cells improve diabetic wound healing. J Invest Dermatol. 2022;142(9):2508-2517.e13.
38. Cui HS, Joo SY, Lee SY, et al. Effect of Hypertrophic Scar Fibroblast-Derived Exosomes on Keratinocytes of Normal Human Skin. Int J Mol Sci. 2023;24(7):6132.
39. Yoo K, Thapa N, Lee J, et al. Dermal fibroblast cell-derived exosomes for atopic dermatitis: In-vitro test. Skin Res Technol. 2023;29(7):e13382.
40. Zhang X, Huang J, Zhao J, et al. Exosome-mimetic vesicles derived from fibroblasts carrying matrine for wound healing. Burns Trauma. 2024:12:tkae015.
41. Eelen G, de Zeeuw P, Treps L, et al. Endothelial cell metabolism. Physiol Rev. 2018;98(1):3–58.
42. Jin W, Li Y, Yu M, et al. Advances of exosomes in diabetic wound healing. Burns Trauma. 2025;13:tkae078.
43. Jiang D, de Vries JC, Muschhammer J, et al. Local and transient inhibition of p21 expression ameliorates age-related delayed wound healing. Wound Repair Regen. 2020;28(1):49–60.
44. Su CW, Lin CW, Yang WE, et al. TIMP-3 as a therapeutic target for cancer. Ther Adv Med Oncol. 2019:11:1758835919864247.
45. Kasolang S, Adlina WA, Rahman NA, et al. Common skin disorders: a review. J Tribol. 2020;25:59–82.
46. Radner FPW, Fischer J. The important role of epidermal triacylglycerol metabolism for maintenance of the skin permeability barrier function. Biochim Biophys Acta. 2014;1841(3):409-15.
47. Burgy O, Königshoff M. The WNT signaling pathways in wound healing and fibrosis. Matrix Biol. 2018:68-69:67-80.
48. Soheilifar MH, Masoudi-Khoram N. Wound dressings incorporating microRNAs: Innovative therapy for diabetic

wound treatment. Iran J Basic Med Sci. 2022;25(9):1042-1044.
49. Zhou S, Zhang P, Liang P, et al. The expression of miR-125b regulates angiogenesis during the recovery of heat-denatured HUVECs. Burns. 2015;41(4):803–11.
50. Blank MF, Grummt I. The seven faces of SIRT7. Transcription. 2017;8(2):67-74.
51. Guo F, Carter DE, Leask A. miR-218 regulates focal adhesion kinase–dependent TGFβ signaling in fibroblasts. Mol Biol Cell. 2014;25(7):1151–8.
52. Miller R, Wormald JCR, Wade RG, et al. Systematic review of fibrin glue in burn wound reconstruction. Br J Surg. 2019;106(3):165-173.
53. Hopfner U, Aitzetmueller MM, Neßbach P, et al. Fibrin Glue Enhances Adipose-Derived Stromal Cell Cytokine Secretion and Survival Conferring Accelerated Diabetic Wound Healing. Stem Cells Int. 2018:2018:1353085.
54. Badekila AK, Kini S, Jaiswal AK. Fabrication techniques of biomimetic scaffolds in three-dimensional cell culture: A review. J Cell Physiol. 2021;236(2):741–62.
55. Zhou H, Xiao J, Wu N, et al. MicroRNA-223 regulates the differentiation and function of intestinal dendritic cells and macrophages by targeting C/EBPβ. Cell Rep. 2015;13(6):1149–60.
56. Tahamtan A, Teymoori-Rad M, Nakstad B, et al. Anti-inflammatory microRNAs and their potential for inflammatory diseases treatment. Front Immunol. 2018;9:1377.
57. Colletti M, Galardi A, De Santis M, et al. Exosomes in systemic sclerosis: messengers between immune, vascular and fibrotic components? Int J Mol Sci. 2019;20(18):4337.
58. Sgonc R, Gruber J. Age-related aspects of cutaneous wound healing: a mini-review. Gerontology. 2013;59(2):159–64.
59. Erlendsson AM, Haedersdal M, Rossi AM. Needle-free injection assisted drug delivery—histological characterization of cutaneous deposition. Lasers Surg Med. 2020;52(1):33–37.