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Mini-Review Article

Therapeutic Potential of Exosomes in Wound Healing with a Focus on Fibroblast-derived Exosomes

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Abstract

Impaired wound healing poses a significant challenge worldwide, impacting patients' quality of life and potentially leading to disability or even mortality. Despite the great progress that has been achieved, it remains a worldwide challenge to develop effective therapeutic treatments for diabetic wounds. Exosomes, have emerged as promising therapeutic tools in wound healing due to their ability to transfer bioactive molecules, including growth factors and cytokines, to target cells. Growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), epidermal growth factor (EGF), and vascular endothelial growth factor (VEGF), play pivotal roles in regulating cellular responses, promoting tissue regeneration, and modulating key signaling pathways. The interplay between growth factors and signaling pathways, including MAPK, PI3K/Akt, and NF-κB, is an important step in wound healing process. In this mini-review we aim to summarize the progress of research on the use of various exosomes derived from different cell types, with a focus on fibroblast-derived exosomes in promoting wound healing.

Keywords: Wound healing, Exosomes, Fibroblasts, Growth factors, Signaling pathways

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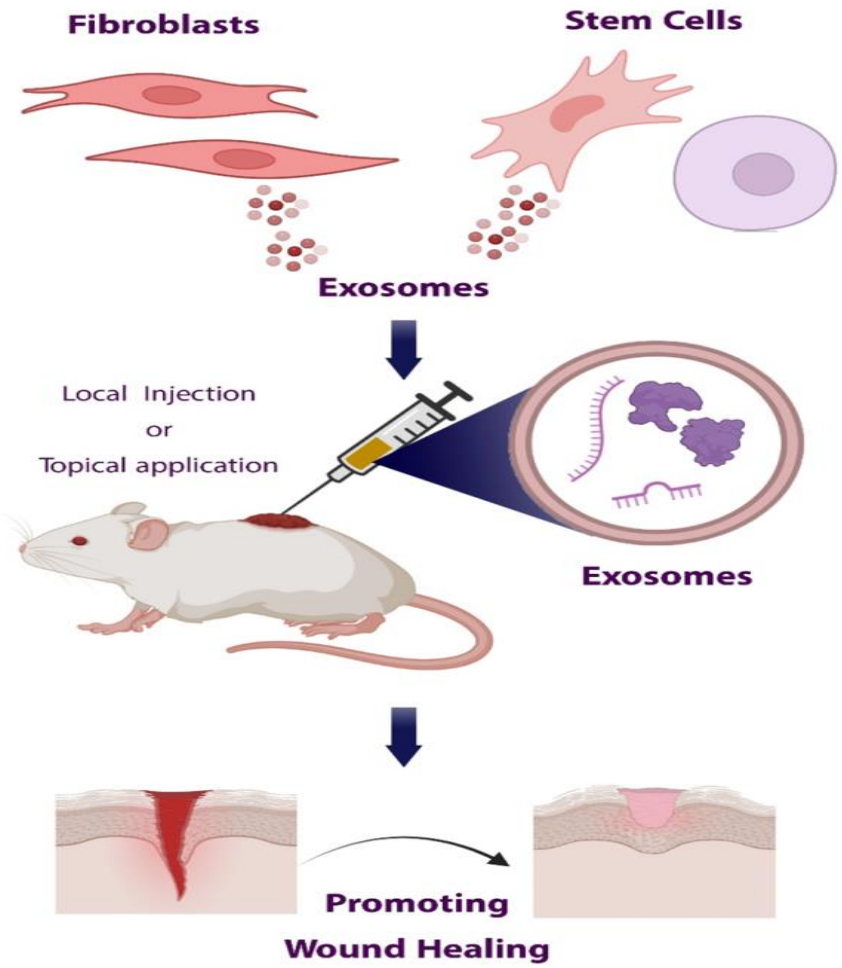

Figure 1. Graphical Abstract

Introduction

Wound healing is a multifaceted and intricate biological process involving various cellular components, complex signaling pathways, and an array of bioactive molecules (1). Successful wound healing is crucial for tissue restoration and re-establishing normal physiological function. However, the global burden of wounds, encompassing both acute and chronic wounds, remains a significant healthcare challenge with far-reaching implications (2). Chronic wounds, a prevalent condition, affect a substantial number of individuals globally, leading to prolonged suffering, compromised quality of life, and significant healthcare costs (3). The search for new therapeutic methods for wound healing has gained considerable attention because of the persistent challenges presented by chronic wounds and the need for more effective interventions (4). Conventional treatments, while beneficial, frequently have limitations when it comes to promoting quick and effective wound healing (4). In recent years, considerable attention has been directed toward exploring the therapeutic potential of exosomes in wound healing (5). Exosomes, small extracellular vesicles encapsulated by a

lipid bilayer membrane, are actively secreted by various cell types. These nano-sized vesicles are rich in proteins, nucleic acids, lipids, and other bioactive molecules, enabling them to serve as potent mediators of intercellular communication and modulators of diverse biological processes (6).

Exosomes have demonstrated remarkable potential in wound healing because of their ability to influence key aspects of the wound repair process. Multiple cell types, including fibroblasts and stem cells, can secrete these extracellular vesicles (7). Exosomes have been shown to promote cell proliferation, migration, and differentiation, thereby speeding up the essential re-epithelialization process required for wound healing (8). In addition, exosomes modulate extracellular matrix remodeling by promoting collagen synthesis and deposition, which are essential for restoring tissue integrity. Moreover, exosomes also have immunomodulatory properties, which influence inflammatory responses resulting in a wound microenvironment suitable for efficient healing (9). In this article, we overview the existing literature concerning the potential utilization of exosomes in wound healing, with a specific focus on fibroblast-derived exosomes.

Stages of Wound Healing

Wound healing comprises an intricate series of biological events involving hemostasis, inflammation, proliferation, and remodeling phases (10).

hemostasis: During this stage, collagen that has been damaged activates the coagulation's intrinsic and extrinsic pathways. A dynamic equilibrium between endothelial cells, thrombocytes, coagulation, and fibrinolysis then defends the vascular system and stops blood loss (11).

Inflammatory: Wound tissue contains neutrophils, monocytes, macrophages, and lymphocytes during this phase. Early and late inflammatory stages comprise this phase. Polymorphonuclear neutrophils (PMN) infiltrate wounds shortly after skin damage to avoid infection. 24-72 hours post-injury. Fixed tissue monocytes activate to produce wound macrophages, which are crucial. The macrophage secretes enzymes and cytokines that activate fibroblasts, keratinocytes, and angiogenesis. Wound macrophages indicate that the late inflammatory phase is ending and healing is entering the proliferative phase (12).

Proliferative: This phase typically begins when hemostasis has been attained and an immune response has effectively established itself in the wound area. This begins on the third day following the initial offense and lasts for approximately two weeks. During the phase of proliferation, wound margin fibroblasts and keratinocytes migrate into the healing zone. The fibroblast synthesizes extracellular matrix (ECM) and serves as a replacement for the network composed of fibrin and fibronectin. As epithelial cells migrate into the incision margins, adjacent keratinocytes are responsible for restoring the epidermis through a process called re-epithelialization (13).

Remodeling: The remodeling phase begins at the ending of granulation tissue evolution and can last for a longer period of time, but typically lasts between 21 days and one year after injury. Along with dermal reorganization and ECM synthesis, the intracellular matrix

matures, collagen bundles increase in diameter, and hyaluronic acid and fibronectin are degraded. Multiple regulators, including growth factors, cytokines, integrins, keratins, matrix metalloproteinases (MMPs), chemokines, and extracellular macromolecules, modulate wound healing, according to the literature. To initiate the restoration process, the cell-cell interaction must be eliminated. Multiple cell types and complex interactions between multiple biochemical cascades are involved in the wound healing process. The discovery of molecular mechanisms in wound healing processes will be a significant turning point in the study of tissue repair (12).

Dynamic Interplay: Growth Factors and Signaling Pathways in Wound Healing

Growth factors, small signaling molecules, have garnered significant attention due to their indispensable functions in promoting cell proliferation, migration, and extracellular matrix synthesis. Among the well-studied growth factors are platelet-derived growth factor (PDGF) (14), transforming growth factor-beta (TGF-β) (15), epidermal growth factor (EGF) (15), and vascular endothelial growth factor (VEGF) (16). These growth factors act through numerous signaling pathways, such as the mitogen-activated protein kinase (MAPK) (17), phosphoinositide 3-kinase (PI3K)/Akt (18), and nuclear factor-kappa B (NF-κB) (19) pathways.

PDGF, a potent mitogen, stimulates fibroblast proliferation and migration, promoting the synthesis of extracellular matrix components and angiogenesis within the wound bed (14). TGF-β regulates cell differentiation, extracellular matrix production, and immune modulation, promoting scar formation and tissue remodeling (15). EGF accelerates re-epithelialization by stimulating keratinocyte migration and proliferation (15). VEGF, a key angiogenic factor, promotes neovascularization, facilitating oxygen and nutrient supply to the wound site (16). The MAPK pathway, including the ERK, JNK, and p38 pathways, plays a pivotal role in cell proliferation, migration, and differentiation during wound healing (17). The PI3K/Akt pathway (18) regulates cell survival, proliferation, and migration, while the NF-κB pathway orchestrates inflammatory responses and mediates cellular events during wound healing (19). The integration of these signaling pathways governs the cellular responses essential for successful wound repair (20).

Exosomes, as crucial mediators of intercellular communication, participate in the dynamic interplay between growth factors and signaling pathways in wound healing. These small extracellular vesicles serve as vehicles for the delivery of growth factors, such as PDGF, TGF-β, EGF, and VEGF, to target cells, modulating cellular responses essential for effective wound repair. By facilitating the transfer of growth factors and their signaling cascades, exosomes contribute to the intricate interplay between growth factors and signaling pathways in orchestrating the complex process of wound healing (21).

Exosomes as Emerging Therapeutic Tools in Wound Healing

In recent years, exosomes have emerged as promising therapeutic agents in the field of wound healing (22). These small extracellular vesicles, secreted by various cell types

including stem cells, fibroblasts, and immune cells, have garnered considerable attention due to their unique properties and ability to modulate cellular responses (23). Exosomes serve as natural carriers of bioactive molecules, including growth factors, cytokines, nucleic acids, and lipids, making them ideal candidates for therapeutic intervention in wound healing (24).

One of the key advantages of exosomes is their ability to transfer their cargo of bioactive molecules to target cells, thereby influencing cellular behavior and promoting wound healing processes (25). Exosomes derived from different cell sources have been shown to enhance wound healing through various mechanisms (26). For example, mesenchymal stem cell-derived exosomes have been reported to stimulate cell proliferation, promote angiogenesis, and modulate the immune response, ultimately contributing to tissue regeneration and improved wound closure (27).

Lee et al. proved that exosomes derived from adipose tissue-derived stem cells (ASCs) can increase cell proliferation and migration in human dermal fibroblasts (HDFs) and also upregulate the expression of genes involved in cell proliferation and wound healing while stimulating collagen production in HDFs (28). In another study, Liu et al. revealed that Human umbilical cord mesenchymal stem cell-derived exosomes promote murine skin wound healing by neutrophil and macrophage modulations (29). MSC-derived exosomes mainly affect skin wound healing by reducing scar formation and myofibroblast accumulation. Furthermore, exosomes can be further engineered for more efficacy, such targeted uptake or contrived enhanced secretion by the specific cells in both in vivo and in vitro condition (30). Some examples of using exosomes with different origins is summarized in table 1.

Exploring the Potential of Fibroblast-Derived Exosomes in Wound Healing

Fibroblast-derived exosomes have emerged as an intriguing area of research with promising implications for wound healing (34, 35). While their specific role and potential therapeutic applications are not yet fully understood, it is widely believed that these exosomes hold significant value in promoting the wound healing process (34). Although limited in number, a recent study conducted in this era has shed light on their potential utility and paved the way for further investigation in this field. Fibroblasts, known for their pivotal role in tissue repair, secrete various growth factors and extracellular matrix components that contribute to the wound healing process (36, 37). As a result, exosomes derived from fibroblasts have attracted attention as potential regulators of cellular communication and wound healing mechanisms (34).

Table1. Some examples of using exosomes with different origins

Host cell	Wound Type	Study Model	Application	Result	Reference
Exo-derived from PRP	Chronic cutaneous wounds	Diabetic Rat Model	PRP-Exos-loaded SAH dressing	Observing of the cutaneous healing process of chronic ulcers by PRP-Exo effect and attributed to YAP activation without species restriction	(31)
hUSCs	full-thickness wounds	Rat	subcutaneously injection	accelerated wound closure and re-epithelization And the angiogenesis of residue tissues were also significantly enhanced.	(32)
hADSCs	full-thickness wound	Balb/c mice	subcutaneous injection	ASCs-Exos recruited to soft tissue wound area in a mouse skin incision model and significantly accelerated cutaneous wound healing	(33)
Adipose Tissue-Derived MSCs	full-thickness wound	SPF micro pigs	subcutaneous injection	ASC-EXOs have beneficial effects on cell proliferation, migration, and gene expression related to wound healing, and they may accelerate wound closure and promote tissue regeneration	(28)
hucMSC	full-thickness wound	-	-	transcriptomic heterogeneity of neutrophils and macrophages in the context of skin wound repair following hucMSC-Exosomes interventions, providing a deeper understanding of cellular responses to hucMSC-Exosomes, a rising target of wound healing intervention	(29)
Fibroblast Cells	full-thickness wound	Rat	topically applied	Utilization of fibroblast-Exo significantly promoted cutaneous wound healing in a rat full-thickness skin ulcer model	(34)

The growth factors are the critical regulatory points of the healing process as they chemo-attract inflammatory cells and fibroblasts to the wound site and cause cellular proliferation (38). Fibroblasts release TGF-β1, which is a pro-fibrotic and pro-migratory growth factor. TGF-β1 stimulates the synthesis of collagen and decreases the degradation of the extracellular matrix (ECM) (38). A reasonable hypothesis is that using fibroblast-exosome at the site of skin injury delivers TGF-β1 and other healing-related factors to the wound milieu and accelerates the repair process. Of note, TGF-β2 carried by exosomes released from fibroblast causes and reduces the proliferation of epithelial cells in severe asthma (39). In a study by Ahmadpour et al. it was shown that utilization of fibroblast-Exo can significantly promote cutaneous wound healing in a rat full-thickness skin ulcer model . In this study, the group treated with high-dose fibroblast-derived exosome (HDE) group showed accelerated healing compared to the negative control (NC) and positive control (PC) groups at 9 and 12 days. Inflammation and granulation were higher in the HDE, LDE, and PC groups than in the NC group ($p < 0.05$). The onset of re-epithelialization and collagen deposition was higher in the low-dose fibroblast-derived exosome (LDE), HDE, and PC groups, then on nine and 12-day, gradually maturing and extending through the ulcer ($p < 0.05$). On day 12, in almost all parameters, the LDE and HDE groups showed improved results compared to NC cases ($p < 0.05$) (34). Results from this study showed that fibroblast-Exo application meaningfully promoted cutaneous wound repair in a rat full-thickness skin ulcer model but it is important to note that further research is required to establish a more comprehensive understanding of the potential usefulness of fibroblast-derived exosomes in wound healing.

Conclusion

Exosomes exhibit unique characteristics that make them suitable for therapeutic applications such as wound healing. Their small size allows for efficient delivery to target tissues and enables them to cross biological barriers. Exosomes also possess inherent stability, protecting their cargo from degradation. Additionally, they display low immunogenicity, reducing the risk of adverse immune reactions, making them attractive candidates for therapeutic interventions in wound healing. The therapeutic potential of exosomes in wound healing has been demonstrated in various preclinical studies and holds promise for clinical translation. Exosomes, including fibroblast-derived exosomes, hold promise as vehicles for delivering growth factors and influencing cellular behaviors involved in wound repair. However, challenges remain in the development and optimization of exosome-based therapies. Standardization of isolation and purification methods, scalability, and the establishment of safety profiles are critical considerations for their successful clinical application. Overcoming challenges and advancing our understanding of these mechanisms will lead to improved therapeutic interventions and better outcomes for individuals with chronic wounds.

Conflicts of interest

The authors confirm that there are no conflicts of interest.

References

1. Bartíková P, Kazimírová M, Štibrániová I. Ticks and the effects of their saliva on growth factors involved in skin wound healing. Journal of venom research. 2020;10:45-52.

2. Riha SM, Maarof M, Fauzi MB. Synergistic Effect of Biomaterial and Stem Cell for Skin Tissue Engineering in Cutaneous Wound Healing: A Concise Review. Polymers. 2021;13(10).

3. Sen CK. Human Wound and Its Burden: Updated 2020 Compendium of Estimates. Advances in wound care. 2021;10(5):281-92.

4. Oryan A, Alemzadeh E, Moshiri A. Burn wound healing: present concepts, treatment strategies and future directions. Journal of wound care. 2017;26(1):5-19.

5. Hettich BF, Ben-Yehuda Greenwald M, Werner S, Leroux JC. Exosomes for Wound Healing: Purification Optimization and Identification of Bioactive Components. Advanced science (Weinheim, Baden-Wurttemberg, Germany). 2020;7(23):2002596.

6. Gao J, Li A, Hu J, Feng L, Liu L, Shen Z. Recent developments in isolating methods for exosomes. Frontiers in bioengineering and biotechnology. 2022;10:1100892.

7. Golchin A, Hosseinzadeh S, Ardeshirylajimi A. The exosomes released from different cell types and their effects in wound healing. Journal of cellular biochemistry. 2018;119(7):5043-52.

8. Joorabloo A, Liu T. Engineering exosome-based biomimetic nanovehicles for wound healing. Journal of Controlled Release. 2023;356:463-80.

9. Zhou C, Zhang B, Yang Y, Jiang Q, Li T, Gong J, et al. Stem cell-derived exosomes: emerging therapeutic opportunities for wound healing. Stem Cell Research & Therapy. 2023;14(1):107.

10. Zhu J, Zhou H, Gerhard EM, Zhang S, Rodríguez FIP, Pan T, et al. Smart bioadhesives for wound healing and closure. Bioactive Materials. 2023;19:360-75.

11. Wang G, Yang F, Zhou W, Xiao N, Luo M, Tang Z. The initiation of oxidative stress and therapeutic strategies in wound healing. Biomedicine & Pharmacotherapy. 2023;157:114004.

12. Beldon P. Basic science of wound healing. Surgery (Oxford). 2010;28(9):409-12.

13. Stechmiller JK. Understanding the role of nutrition and wound healing. Nutrition in clinical practice. 2010;25(1):61-8.

14. Jian K, Yang C, Li T, Wu X, Shen J, Wei J, et al. PDGF-BB-derived supramolecular hydrogel for promoting skin wound healing. Journal of Nanobiotechnology. 2022;20(1):1-9.

15. Bernabé-García Á, Liarte S, Rodríguez-Valiente M, Nicolás FJ. Chronic wound healing by amniotic membrane: TGF-β and EGF signaling modulation in re-epithelialization. Frontiers in bioengineering and biotechnology. 2021;9:689328.

16. Bao P, Kodra A, Tomic-Canic M, Golinko MS, Ehrlich HP, Brem H. The role of vascular endothelial growth factor in wound healing. Journal of Surgical Research. 2009;153(2):347-58.

17. Yew T-L, Hung Y-T, Li H-Y, Chen H-W, Chen L-L, Tsai K-S, et al. Enhancement of wound healing by human multipotent stromal cell conditioned medium: the paracrine factors and p38 MAPK activation. Cell transplantation. 2011;20(5):693-706.

18. Jere SW, Houreld NN, Abrahamse H. Role of the PI3K/AKT (mTOR and GSK3β) signalling pathway and photobiomodulation in diabetic wound healing. Cytokine & Growth Factor Reviews. 2019;50:52-9.

19. Ambrozova N, Ulrichova J, Galandakova A. Models for the study of skin wound healing. The role of Nrf2 and NF-κB. Biomedical Papers of the Medical Faculty of Palacky University in Olomouc. 2017;161(1).

20. Wang Y, Zhu J, Chen J, Xu R, Groth T, Wan H, et al. The signaling pathways induced by exosomes in promoting diabetic wound healing: a mini-review. Current Issues in Molecular Biology. 2022;44(10):4960-76.

21. Geiger A, Walker A, Nissen E. Human fibrocyte-derived exosomes accelerate wound healing in genetically diabetic mice. Biochemical and biophysical research communications. 2015;467(2):303-9.

22. Zhao W, Zhang H, Liu R, Cui R. Advances in Immunomodulatory Mechanisms of Mesenchymal Stem Cells-Derived Exosome on Immune Cells in Scar Formation. International Journal of Nanomedicine. 2023:3643-62.

23. Tienda-Vázquez MA, Hanel JM, Márquez-Arteaga EM, Salgado-Álvarez AP, Scheckhuber CQ, Alanis-Gómez JR, et al. Exosomes: A Promising Strategy for Repair, Regeneration and Treatment of Skin Disorders. Cells. 2023;12(12):1625.

24. Ahn S-H, Ryu S-W, Choi H, You S, Park J, Choi C. Manufacturing therapeutic exosomes: from bench to industry. Molecules and Cells. 2022;45(5):284.

25. Di Bella MA. Overview and update on extracellular vesicles: Considerations on exosomes and their application in modern medicine. Biology. 2022;11(6):804.

26. Hu L, Wang J, Zhou X, Xiong Z, Zhao J, Yu R, et al. Exosomes derived from human adipose mensenchymal stem cells accelerates cutaneous wound healing via optimizing the characteristics of fibroblasts. Scientific reports. 2016;6(1):32993.

27. Qiu H, Liu S, Wu K, Zhao R, Cao L, Wang H. Prospective application of exosomes derived from adipose-derived stem cells in skin wound healing: A review. Journal of cosmetic dermatology. 2020;19(3):574-81.

28. Lee JH, Won YJ, Kim H, Choi M, Lee E, Ryoou B, et al. Adipose Tissue-Derived Mesenchymal Stem Cell-Derived Exosomes Promote Wound Healing and Tissue Regeneration. International Journal of Molecular Sciences. 2023;24(13):10434.

29. Liu Y, Zhang M, Liao Y, Chen H, Su D, Tao Y, et al. Human umbilical cord mesenchymal stem cell-derived exosomes promote murine skin wound healing by neutrophil and macrophage modulations revealed by

single-cell RNA sequencing. Frontiers in Immunology. 2023;14.

30. Fang S, Xu C, Zhang Y, Xue C, Yang C, Bi H, et al. Umbilical Cord-Derived Mesenchymal Stem Cell-Derived Exosomal MicroRNAs Suppress Myofibroblast Differentiation by Inhibiting the Transforming Growth Factor-β/SMAD2 Pathway During Wound Healing. Stem cells translational medicine. 2016;5(10):1425-39.

31. Guo S-C, Tao S-C, Yin W-J, Qi X, Yuan T, Zhang C-Q. 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.

32. 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.

33. Zhang J, Guan J, Niu X, Hu G, Guo S, Li Q, et al. Exosomes released from human induced pluripotent stem cells-derived MSCs facilitate cutaneous wound healing by promoting collagen synthesis and angiogenesis. Journal of translational medicine. 2015;13:49.

34. Ahmadpour F, Rasouli HR, Talebi S, Golchin D, Esmailinejad MR, Razie A. Effects of exosomes derived from fibroblast cells on skin wound healing in Wistar rats. Burns : journal of the International Society for Burn Injuries. 2023.

35. Hsu H-H, Wang AYL, Loh CYY, Pai AA, Kao H-K. Therapeutic potential of exosomes derived from diabetic adipose stem cells in cutaneous wound healing of db/db mice. Pharmaceutics. 2022;14(6):1206.

36. Madsen SF, Sand JMB, Juhl P, Karsdal M, Thudium CS, Siebuhr AS, et al. Fibroblasts are not just fibroblasts: clear differences between dermal and pulmonary fibroblasts' response to fibrotic growth factors. Scientific Reports. 2023;13(1):9411.

37. Luo Y, Kobler JB, Zeitels SM, Langer R. Effects of growth factors on extracellular matrix production by vocal fold fibroblasts in 3-dimensional culture. Tissue Engineering. 2006;12(12):3365-74.

38. Sorg H, Tilkorn DJ, Hager S, Hauser J, Mirastschijski U. Skin Wound Healing: An Update on the Current Knowledge and Concepts. European surgical research Europaische chirurgische Forschung Recherches chirurgicales europeennes. 2017;58(1-2):81-94.

39. Haj-Salem I, Plante S, Gounni AS, Rouabhia M, Chakir J. Fibroblast-derived exosomes promote epithelial cell proliferation through TGF-β2 signalling pathway in severe asthma. Allergy. 2018;73(1):178-