CellR4 2023; 11: e3402 DOI: 10.32113/cellr4_20237_3402

Application of stem cells and adipose-derived stem cell exosomes on dermal wound healing

D. Arbabha[1], A. Yalaza[2], N. Başaran[3], P. Koçak[4]

[1]Cagaloglu Anatolian High School, Istanbul, Turkey
[2]VKV Koc High School, Istanbul, Turkey
[3]Stem Cell and Tissue Engineering, Istinye University, Istanbul, Turkey
[4]Biomedical Engineering, Istinye University, Istanbul, Turkey

Corresponding Author: Polen Kocak, MD; e-mail: polen.kocak@istinye.edu.tr

Keywords: Stem cells, Exosomes, Dermal wound healing, Differentiation, Stem cell potency.

Abstract – The skin is the largest organ in the human body and plays a significant role in protecting the body from external threats, containing tissues that sustain the body's homeostasis. Wound healing in the skin is a complex process involving the interaction of various cell types in the target tissue, including but not limited to cytokines and growth factors. In extreme cases, disorders of the cardiorespiratory system, such as chronic diseases, may prevent the process of wound healing entirely. Numerous studies have been conducted to discover methods to restore the ability of chronic wounds to cure themselves, but this remains one of the most significant medical problems. Exosomes derived from stem cells have been extensively proposed as a treatment for dermal wound recovery. Different types of stem cells have varying therapeutic potential. Exosomes, a component of paracrine, have the function of enhancing the effectiveness of stem cells. This article discusses the wound healing process as well as the mechanism of stem cell and adipose-derived exosome therapy on cutaneous wound healing and its clinical applications.

Introduction

The skin, the largest organ in the body, consists primarily of two layers: the epidermis and the dermis. Dermal wounds are injuries to the dermal layer of skin tissue caused by procedures, accidents, burns, abrasions, or chronic conditions such as diabetes. Two types of dermal lesions are distinguished: acute and chronic. Acute wounds undergo a structured healing process, whereas chronic wounds may exhibit irregularities due to underlying conditions. In healthy patients, wound healing occurs following any skin tissue injury. It is a lengthy procedure with numerous phases and conditions. Many patients with chronic diseases, such as those with diabetes or a compromised metabolism, have difficulty repairing incisions, as is the case with diabetes patients and patients with a weakened metabolism. Due to the difficulty of curing incisions in these patients, wounds may go as far as resulting in amputations[1].

Stem cells are capable of differentiating into various cell types and regenerating themselves. Each form of stem cell has its own distinct advantages and disadvantages. This review will discuss the characteristics of embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), bone marrow-derived mesenchymal stem cells (BM-MSCs), and adipose-derived mesenchymal stem cells (ASCs)[2].

Exosomes are the smallest extracellular vesicle population. They are enveloped by a bilayer of lipids and contain proteins, lipids, mRNA, and DNA derived from the original cell.

Due to their small sizes, which are 30-150 nm in diameter, they can readily penetrate various cells and can be released into the extracellular environment. Exosomes derived from adipose-derived mesenchymal stem cells (ASCs-EXOs) can modulate wound healing by regulating immune

responses and the inflammatory process. They enhance epidermis cell angiogenesis, proliferation, and re-epithelialization. By modulating collagen remodeling, ASCs-EXOs have the unique ability to restrict scar hyperplasia, thereby creating the possibility of dermal wound healing without a scar. In clinical applications, ASCs-EXOs are capable of facilitating adipose transplantation and enhancing wound healing in patients with chronic diseases such as diabetes[1,2].

Dermal Wound Healing

Dermal wound healing involves the interactions between cells such as fibroblasts, keratinocytes,and endothelial cells. Neutrophils, monocytes, macrophages, lymphocytes, and dendritic cells are also involved as immune components. In this review, dermal wound healing will be examined through four main stages: homeostasis, inflammation, proliferation, and remodeling. Homeostasis entails all metabolic activities that maintain the equilibrium between various factors, such as hormone levels, epidermis conditions, and the overall health of the patient. Fibrin and blood platelets form a blood clot during the inflammatory process to prevent further blood and fluid loss and to create a barrier against inflations. During this phase, the damaged tissue initiates the release of histamine, which causes vasodilation and increases the permeability of blood vessels in the tissue, allowing white blood cells to enter the wound. Through phagocytosis, these white blood cells safeguard the lesion from foreign particles[3].

Following a period of two to ten days, cellular proliferation results in the formation of a novel tissue layer that covers the wound. The formation of the scab results in the initial creation of tissue after the exterior of the clot has dried. The cellular composition of this particular tissue comprises fibroblasts, myofibroblasts, and endothelial cells. Macrophages play a crucial role in orchestrating the response to tissue injury by secreting macrophage-derived growth factor (MDGF) to stimulate the proliferation of fibroblasts and endothelial cells. Fibroblasts infiltrate the wound and secrete collagen, strengthening the clot. The conglomeration of cells collaboratively substitutes the clot and generates the granulation tissue[4]. The duration of the remodeling phase typically ranges from three weeks to six months. During this phase, the removal of the scab takes place, leading to the reorganization of collagen fibers and the restoration of blood vessels to their normal state. Throughout this procedure, there is a reduction in the number of fibroblasts. The process of fibrosis involves the replacement of the damaged tissue with scar tissue. The scar tissue exhibits a higher concentration of collagen fibers, resulting in decreased elasticity[3,4].

Present Therapeutic Strategies Used in Wound Healing

Multiple physiological and cellular mechanisms are used during the complicated and dynamic process of wound healing to rebuild injured tissue. There are many therapy procedures utilized in wound healing to achieve optimal healing and reduce scarring. One of the frequently employed techniques is wound debridement, which removes dead tissue and encourages tissue regeneration. One of the other techniques is wound dressings, which regulate moisture and encourage a moist environment. Another technique for wound healing is growth factor and cytokine therapy, which stimulates cell migration and proliferation. Pressure wound therapy is also used in wound healing by encouraging blood flow and eliminating infectious material[5]. The use of antimicrobial drugs to stop infections, hyperbaric oxygen therapy to enhance oxygenation, and electrical stimulation to assist tissue healing are some other methods. In more extreme situations, surgery could be required to treat underlying issues that are preventing wound healing. A multidisciplinary team approach including a wound care specialist, physical therapist, and surgeon is crucial for optimal wound healing since each wound healing case is unique and requires a specialized approach to obtain the greatest results[6,7].

Stem Cells (SCs)

Stem cells are a class of cells that have long-term self-renewal ability and can differentiate into other types of cells that are more specialized within their functions. Through the differentiation process, the DNA does not vary between mother and daughter cells, however, the gene expression varies in each

specialized cell. Stem cells are found in almost every adult organ and are responsible for replacing the cells that are lost within these organs and responding to any injury or disease in the tissue. Through their differentiation pathways, there are intermediate or progenitor states. These progenitor cell states can influence the behavior of cells around them without differentiating into tissue cell types. Due to these properties, stem cells have been widely used in tissue engineering and cell therapies. Stem cells can be engineered and modified *in vitro* to differentiate into desired cells[8].

The potential of stem cells to differentiate into specialized cell types is known as stem cell potency. Potency defines the ability of stem cells to adopt a different phenotype or differentiate. Stem cells can be identified by their potencies, such as totipotent, pluripotent, and multipotent. Totipotent stem cells are formed in low amounts in the zygote. These stem cells can differentiate into every cell type in the body and the placenta.Pluripotent stem cells are found in the blastocyst and can differentiate into all body cell types other than the placenta. Multipotent stem cells are more specialized and are found in the ectoderm, endoderm, and mesoderm. They differentiate into different cell types according to the germ layer that they originate from. Unipotent stem cells exhibit long-term self-renewal and can reproduce in large amounts. They have the ability to differentiate into one specific cell type[9].

Stem cells have a high interest in the cancer field thanks to their role in the development, growth, and recurrence of tumors. Cancer stem cells, a subgroup of tumor cells with stem cell-like characteristics, have been linked to tumor recurrence and resistance to standard cancer treatments. Because of stem cells' capacity to specialize in a variety of cell types, stem cell-based cancer therapies have been created, including the use of hematopoietic stem cell transplantation to treat specific types of blood malignancies. The potential of mesenchymal stem cells to target and alter the tumor microenvironment, enhancing immune system activation and minimizing inflammation, has also been studied[8]. Although stem cell therapy for cancer is still in its infancy, it offers a potential path for creating novel and potent cancer medicines. However, to completely comprehend the mechanisms underlying cancer stem cell therapy, and to create secure and efficient clinical application procedures, more investigation is required[8,9].

Stem Cells in Wound Healing

Stem cells (SCs) can be differentiated into the desired cell type *in vitro,* and thus their regenerative abilities can be induced. The regenerative abilities of stem cells are based on the production of growth factors, immunomodulators, and external bioactive molecules apparent in the extracellular vesicles. Stem cells can promote the secretion of regenerative cytokines. Due to their pluripotency, self-renewal abilities, and capacities to promote the secretion of regenerative cytokines, stem cells have become widely used in cell therapies. Stem cells have been proposed[10]. to advance dermal wound healing by controlling the activity of macrophages, T cells, and B cells, decreasing inflammation, and secreting vascular endothelial growth factors (VEGF), advancing angiogenesis. SCs promote the multiplication and differentiation of fibroblasts and keratinocyte-forming cells, create cytokines against fibrosis and have the ability to differentiate into microvascular endothelial cells and other keratinocytes[11].

Embryonic Stem Cells (ESCs)

Embryonic stem cells (ESCs) can be found in the inner blastocyst. ESCs can differentiate into three primary germ layers: ectoderm, mesoderm, and endoderm. Thus, ESCs are categorized as pluripotent stem cells. ESCs can be differentiated into every mature specialized cell type with *in vitro* studies. Studies[12] have also successfully differentiated ESCs into keratinocytes, which are one of the key cellular components of the epidermis and are used for the reconstitution of multilayers. However, there are many legal and ethical concerns regarding ESCs, due to the issues regarding the harvest of these cells from the embryo, and the difficulty of isolating them. Also, due to their high self-renewal and pluripotency, there is the risk of tumor development from the clinical application of ESCs. In addition to these limitations, it should be known that there is a risk in cell therapy within allogeneic applications for patients because of the possibility of rejection[13].

Induced Pluripotent Stem Cells (iPSCs)

Induced pluripotent stem cells (iPSCs) were first developed to overcome the ethical and practical concerns associated with ESCs. These cells are

derived from de-differentiated somatic cells, and have similar properties to ESCs, regarding their self-renewal and pluripotent abilities. iPSCs have the advantage of ethical harvesting and do not concern human embryos. Regarding dermal wound healing, research has found that human iPSCs successfully differentiate into endothelial cells and keratinocytes, which can promote wound healing. However, there have been many safety risks found regarding the therapeutic application of these cells, such as cancer being seen in some chimeric mice. More research and improvements are needed until iPSCs can effectively and safely be used for clinical applications of regenerative therapy[14].

Bone Marrow-Derived Mesenchymal Stem Cells (BM-MSCs)

Mesenchymal stem cells (MSCs) are multipotent, regenerative and differentiate into different cell types, specifically forming tissues such as skin cells. MSCs can be harvested from the patient's bone marrow, adipose tissue, nerve tissue, umbilical cord blood, or dermis. MSCs have fewer ethical and practical problems, compared to other types of stem cells like iPSCs, and ESCs. They are relatively easier to access and isolate and have a very low risk of rejection from the patient's body after the case of transplant, due to their low immunogenic potentials. Regarding their regenerative abilities on dermal wound healing, bone marrow-derived mesenchymal stem cells (BM-MSCs), and adipose-derived mesenchymal stem cells (ASCs) have been the most studied stem cells in the literature[15].

Previous studies[16] have indicated that BM-MSCs can stimulate the upregulation of collagen, fibroblast growth factors, and endothelial growth factors, thereby augmenting the dermal wound healing process. Beyond growth factors, bone marrow-derived mesenchymal stem cells (BM-MSCs) have demonstrated efficacy in enhancing the wound healing process through the promotion of epithelialization and angiogenesis. Additionally, BM-MSCs possess the capacity to differentiate into specialized epithelial cells that express keratinocytes. Research has demonstrated the efficacy of employing BM-MSCs in the process of wound healing in both human and murine subjects. Although the outcomes have been favorable, the requirement to generate substantial quantities of cell culture for efficacious medical treatment may pose a challenge for extensive wounds on a large scale. Moreover, it has been reported that the efficacy of BM-MSCs therapy diminishes with advancing age[15].

Adipose-Derived Stem Cells (ASCs)

Adipose-derived stem cells (ASCs) have become the most widely used stem cell in cell therapies and tissue engineering. The process of harvesting, accessing, and distributing them to patients is relatively simple. These cells possess the capacity to undergo differentiation into adipogenic, chondrogenic, myogenic, and osteogenic lineages. Studies[17] have found that 500 more stem cells could be acquired from adipose tissue compared to bone marrow tissue. In contrast to BM-MSCs, ASCs are comparatively more accessible, exhibit a delayed onset of senescence, and possess a greater capacity for proliferation. Adipose-derived stem cells (ASCs) are additionally engaged in the autocrine synthesis of growth factors and immunomodulatory agents[18].

Although ASCs have only recently been discovered, they have gained rapid popularity and have already been utilized in various clinical therapies. They have been especially successful in regenerative cell therapy, mainly being used for reconstructive and cosmetic purposes. Numerous *in vivo* and *in vitro* experiments have exhibited the potential of ASCs in the context of dermal wound healing. Research has indicated that Adipose-derived stem cells (ASCs) possess the capacity to speed up re-epithelization in dermal wounds. This is achieved through the activation of fibroblasts *via* cell-to-cell interactions and/or the secretion of various growth factors in a paracrine manner. Autologous stem cells (ASCs) have the potential to be administered *via* direct injection into the patient's body or generated through *in vitro* tissue engineering techniques. In short, ASCs can contribute to the process of dermal wound healing by actively creating growth factors and promoting angiogenesis and the proliferation of keratinocytes[19].

Nevertheless, there are still certain risks associated with ASCs. Due to their high self-renewal and differentiation abilities, they still have the risk of tumor growth and metastases. The efficacy of adipose-derived stem cells (ASCs) in cell therapy is significantly influenced by the physiological condition of the recipient, including factors such as age,

gender, and body mass. Despite the existing studies[18,19] and practical implementations, additional extensive investigations are warranted to mitigate the residual hazards and challenges associated with these cellular therapies.

Cell-Free Exosomes

Exosomes are small, secreted organelles surrounded by a single membrane. They contain a selected group of proteins, lipids, nucleic acids, and glycoconjugates. In the cell, they are available in the nucleus and cytoplasm and take part in RNA processing. Exosomes can be secreted by B and T cells, dendritic cells, mesenchymal stem cells, epithelial and endothelial cells, and cancer cells. Exosomes are capable of remodeling the extracellular matrix and transmitting signals and molecules between cells once they are released from the host cell. Due to their extremely small proportions, they are able to access and leave compartments. This cell-to-cell interaction plays a significant role in human metabolism and health, including development, immunity, the maintenance of homeostasis, the onset of malignancy, and the development of numerous diseases. Viruses and other evading particles can use these vesicle pathways to spread their infections. Thanks to their targetable activity and ability to cross the blood-brain barrier, they can also form an excellent drug delivery pathway[20].

Exosomes play a crucial role in paracrine signaling and are the primary determinant of stem cell efficacy. The use of cell-free exosome therapy can overcome numerous drawbacks of stem cells, such as their stability and storage convenience. They are highly biocompatible, do not carry the risk of being rejected by the host's body, and can be readily dose-controlled[20].

ASCs-Derived Exosomes in Wound Healing

Inflammation issues caused by chronic or excessive inflammation can hinder the wound-healing process. Exosomes derived from adipose-derived stem cells (ASCs-EXOs) have the capacity to modulate inflammation by inhibiting the immune system. In addition, they contain immunoregulatory proteins that support this stage. ASCs-EXOs accelerate the proliferation and migration of vascular endothelial cells during the angiogenesis phase. During proliferation, the dosage of ASCs-EXOs determines their effectiveness. These exosomes can enter fibroblasts and stimulate their proliferation, migration, and collagen production. In addition, ASCs-EXOs have the ability to regulate collagen remodeling, thereby inhibiting scar hyperplasia. This characteristic makes ASCs-EXOs ideal for potential clinical applications involving scar-free wound recovery. However, there is still room for additional research in this discipline[21].

Fat grafting or lipo-modeling is a cosmetic surgery procedure in which undesirable fat is extracted from one area of the body and injected into another, more desirable area. An adequate quantity of angiogenesis is required for a successful fat transplantation procedure. As previously mentioned, ASCs-EXOs are capable of promoting angiogenesis by stimulating the proliferation and migration of vascular endothelial cells. Researchers have discovered[21] that ASCs-EXOs can enhance the wound-healing capabilities of diabetic rodents by promoting angiogenesis, proliferation, and the synthesis of collagen and fibroblasts in the lesion. There have also been reports of the efficacy of ASCs-EXOs in diabetic foot wound healing.

Plant Exosomes in Dermal Wound Healing

Plant cells secrete tiny vesicles called exosomes, which have been revealed to have potential therapeutic benefits in several medical fields. According to recent studies, plant exosomes may also aid in the healing of dermal wounds. Bioactive substances like microRNAs, which can control the expression of genes in recipient cells, are found in plant exosomes. Plant exosomes have been demonstrated to speed up wound closure and improve healing outcomes by boosting dermal cell migration and proliferation when administered to the wound site. Additionally, by regulating the immunological response and regulating fibroblast activity, plant exosomes can lessen inflammation and scarring. These results sign that plant-derived therapeutics may represent a promising route for the development of novel wound healing treatments, even if further study is required to completely understand the processes underlying the therapeutic benefits of plant exosomes on wound healing[22,23].

Conclusions and Future Trajectories

The clinical application of stem cells and exosomes is fairly new in the field of regenerative therapy. These technologies have the potential to resolve non-healing wounds in chronic patients, a medical problem that has been a burden for these patients throughout history. However, despite all of the encouraging results, cell therapies have remained controversial. There are many ethical issues regarding the harvest of stem cells, such as embryonic and umbilical-cord stem cells. There is also the risk of tumor growth and metastatic behavior of the tissue created by the stem cell, due to the high senescence and self-renewal potential of stem cells. Biocompatibility is also an issue regarding allogeneic cell therapies, because of the risk of the patient's body rejecting the injected therapy.

Cells are living creatures; thus, they are highly unstable, and it is difficult to create a structured therapy that can fit every patient. There are many legal procedures that need to be followed in order to commercialize cell therapies. Organizations like the Food and Drug Administration (FDA) in the United States and the European Medicines Agency in Europe regulate the research, manufacturing, and clinical application process of these therapies, and it is important that cell therapies can be able to fit into the structural perquisites of becoming an accepted technology.

Due to having regenerative properties despite being cell-free, exosomes have recently become a focal point in the area of regenerative therapy. Because they are not afflicted with cells, they are highly biocompatible and stable, and their contents and dosages can be relatively easy to control. They do not contain the risk of creating tumors or being rejected by patients after the therapy. These properties make exosomes easier to regulate and apply in larger clinical settings.

Conflict of Interest:
The authors declare no conflicts of interest with respect to the authorship and/or publication of this manuscript.

Funding:
The authors received no financial support for the research and/or authorship of this manuscript.

Authors' Contributions:
P.K., N.B., D.A., and A.Y. wrote the manuscript, N.B., D.A., and A.Y. proceeded with proofreading. P.K. provided feedback on the study and checked the manuscript during the submission. All authors have given approval to the final version of the manuscript.

Ethics Approval and Informed Consent:
Not applicable.

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