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Exosome Therapy in Combination with Photodynamic Therapy for Severe and large-scale Injuries and Resisted Wound Treatment: Case Series

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Abstract **Original Research Article**

***Background*:** There are many scientific studies today showing the role of PDT in wound healing. Also, one of the last progressive methods for healing purposes is applying exosomes. ***Objectives*:** We investigated the healing process of sever and large-scale injuries and resisting wounds following riboflavin-mediated photodynamic therapy (PDT) that can easily disinfect the wound region in cellular and sub-cellular levels. On the other side, exosomes are one of the most rapid growing methods for regenerative purposes. ***Materials and Methods*:** Five subjects with large surface wounds and injuries were treated with PDT (laser irradiation 375 nm and 447 nm, 10 J/cm2, 100 mW) in combination with autologous exosome derived from adipose tissue. Major changes in the wound area have been illustrated. After PDT, 10 billion (in 5ml volume) of adipose derived exosome were applied on a wound trans-dermally. ***Results*:** All cases had clinical improvement in the wound area after two weeks without surgery. ***Conclusion*:** PDT in combination with exosome therapy can be applied on a large scale and resistant infected wounds as a complementary medicine.

Keywords: photodynamic therapy (PDT), wound healing, wound trans-dermally, exosome therapy.

INTRODUCTION

Wound healing is a complex process in response to trauma, aimed at restoring skin integrity. It is involved in the chronological and balanced activity of inflammatory cells, blood vessels, connective tissue, and epithelium. Various methods have been advocated to speed up this process and avoid excessive scar formation (Weber, Ghanbarzadeh, *et al.*, 2023; Weber, Mehran, *et al.*, 2023). The use of low-intensity light therapy (LLLT) (Hamblin *et al.*, 2016; Hamblin & Huang, 2013; Tuner & Hode, 2004), stem cell therapy and exosome therapy (Kucharzewski *et al.*, 2019) (Kosaric *et al.*, 2019) (Rani & Ritter, 2016) plus vitamin therapy (Sinno *et al.*, 2011) has been advocated, however, in large-scale wounds there is a risk of infection or complications changed to cancer, PDT is more promising (Weber, Ghanbarzadeh, *et al.*, 2023; Weber, Mehran, *et al.*, 2023).

Wound healing (Weber, Ghanbarzadeh, *et al.*, 2023) is a process that requires a complex interaction of resident mesenchymal and epithelial cells to perform the four steps of the process: hemostasis, inflammation, re-epithelialization, and regeneration. These stages spatially overlap and interact in complex ways to ultimately complete the healing process. Wound healing is an active area of clinical research, particularly regarding factors that improve the speed or quality of wound healing. It is essential to identify effective, rapid and economical treatments without side effects to improve wound healing (Nesi-Reis *et al.*, 2018; Oyama *et al.*, 2020; Yang *et al.*, 2021).

Photodynamic therapy (PDT) is a process in which light interacts with a photosensitive molecule, in the presence of oxygen, to produce reactive oxygen species that will destroy host cells. Although PDT is mainly applied in the cancer setting, it has also been widely used for vascular targeting in age-related macular degeneration and is currently under investigation for microbial activity. its, especially in wound or

periodontitis. Photodynamic therapy (PDT) has been used to treat cancer, infectious diseases, and inflammatory conditions, including acne, rosacea, genital warts, and others. Skin irradiation to perform PDT after systemic use of photosensitizers known to cause skin necrosis. Recent studies have also reported that topical application of photosensitizers and repeated illumination improved wound healing (Hamblin & Huang, 2013; Oyama *et al.,* 2020; Weber, Ghanbarzadeh, *et al.,* 2023; Yang *et al.,* 2021).

Photosensitive riboflavin is one of the strongest generation photosensitizers that has many forms of application such as intravenous or oral dormitories or transdermal like a cream (Maisch *et al.,* 2014). PDT uses a combination of a material, called a photosensitizer, and specific wavelengths of harmless light, which in turn produce photochemical reactive oxygen species (ROS) that affect cell signaling and induce selective cell death or injury in target tissues as well as microorganisms and unhealthy cells. The major products of photochemical activation are ROS, which are involved in the regulation of intracellular signal transduction in vivo. Although the exact details of the regulatory process have not been elucidated, the target signaling systems of ROS are from the cell surface to the nucleus (Arboleda *et al.,* 2014; Etemadi *et al.,* 2021; Maisch *et al.,* 2014).

The dose of PDT is an important factor in determining the concentration of ROS in photochemical reactions (Arboleda *et al.,* 2014). Unlike cytotoxicity induced by high concentrations of ROS, low dose PDT affects proliferation and differentiation without significantly increasing cell death, thereby promoting differentiation of cells and pluripotent stem cells, such as mesenchymal stem cells, osteoblast progenitor cells, neural stem cells, and others (Arboleda *et al.,* 2014).

Modulatory effects of exogenous ROS on stem cells have been demonstrated in vitro. Recently, a study demonstrated that topical ROS production in rat skin activated hair follicle stem cell proliferation, stimulated resting phase hair growth, and promoted wound healing due to burns. However, little is currently known about the exact effects of exogenous ROS produced by photoactive activation on the epidermis during wound healing. Several studies demonstrate that doses of PDT lower than those traditionally used to kill cancer cells can induce sufficient ROS to stimulate epidermal cells, both in vivo and in vitro, of low-dose PDT improve the differentiation and migration of the epidermis, which is helpful for the healing process, without significant cytotoxicity, by promoting epithelial regeneration, angiogenesis, and regulation of inflammation (Etemadi *et al.,* 2021; Huang *et al.,* 2009).

Also, exosomes due to their unique characteristics have attracted the attention of researchers to exploit them as biomarkers in the diagnosis and monitoring of various diseases. Exosomes are 40–150 nm extracellular vesicles with a double-layer membrane secreted by cells and contain elements such as nucleic acids, lipids, and proteins. These vesicles exhibit characteristics such as low immunogenicity, innate stability, good membrane penetration, biocompatibility, regeneration, and immunomodulation. It has been hypothesized that exosome-based therapies are more effective, safer, less cumbersome, and require less equipment and preparation time than stem cells for therapeutic purposes. Therefore, treatments using stem cells, especially exosomes derived from them, may contribute to more effective treatment of large-scale wounds.

The primary mechanism by which exosomes play a role in therapy is paracrine effects, and exosomes are the main contributors to these paracrine effects. Since the main therapeutic properties of Mesenchymal Stem cells (MSCs) depend on their paracrine effects, it appears that MSC-derived exosomes may also provide an alternative option to promote wound healing and regeneration skin as a cell-free innovation. Such exosomes transport functional substances (e.g. growth factors, cytokines, miRNAs, etc.) from MSCs to target cells, thereby influencing the biological activities of recipient skin cells, e.g. such as migration, proliferation and secretion of collagen.

In this study we used PDT to treat large scale damaged tissues and wounds by this method. In our previous studies, like this study, we saw extraordinarily good results by laser or laser in combination with photosensitizers in wounds. For better repairing procedures, we applied at least 10 billion of exosomes after PDT.

MATERIAL AND METHODS

Cases

Five patients with chronic wounds and repeated unsuccessful and recurrent wound recurrences participated in the study. All of them were the case for a surgery by medical committee agreements.

Isolation of Human Adipocyte-Derived Mesenchymal Stromal Cells (HADMSC)

Adipose tissue samples were obtained from healthy autologous donors; Written informed consent was obtained from all patients involved according to the guidelines of the Declaration of Helsinki. Human liposuction from patients undergoing selective aspiration-assisted lipectomy will be collected; Additionally, donor blood samples were negative for viral infections, including mycoplasma, cytomegalovirus (CMV), hepatitis B virus (HBV), hepatitis C virus (HCV), and hepatitis B virus (HCV). human (HIV) immunity, assessed by polymerase chain reaction (PCR). Tissues were obtained using aseptic technique and stored in Hanks' balanced salt solution (HBSS), free of Ca and Mg with 1% (v/v) penicillin/streptomycin. To isolate ASC, approximately 5 g of adipose tissue was minced

and washed twice with PBS buffer, followed by centrifugation at 1,200 rpm for 10 min. The supernatant was discarded; The extracellular matrix was digested with 0.2% collagenase II for 2 h at 37°C on a shaker to release the cellular fractions. After digestion, complete medium was added to stop the reaction and the digested mixture was filtered through a 40 mm cell filter and centrifuged at 1200 rpm for 10 min. The supernatant was removed after centrifugation and the resulting pellet was resuspended in DMEM (DMEM) supplemented with 10% (v/v) human serum (human serum was obtained from plasma collected from the patient). and 1% (v/v) penicillin/streptomycin. Isolated cells were grown as monolayers in T175 flasks using low-glucose DMEM at 37°C in a humidified atmosphere containing 5% CO_2. Immunophenotypic analysis: to analyze the immunophenotype of expanded cells, cells were detached and washed with phosphate-buffered saline (PBS) containing bovine serum albumin and incubated. with primary antibodies against human (CD73, CD 105, CD90 and CD 44; and negative for hematopoietic markers (CD45, CD 34 and HLA-DR). After washing with PBS containing bovine serum albumin, cells were incubated with fluorescein isothiocyanate (FITC) and phycoerythrin (PE)-conjugated secondary antibody. After three washes, cells were resuspended in PBS and analyzed by flow cytometry using a FACSCalibur flow cytometer.

Procedure

Riboflavin-5-phosphate (100 mg capsule) was provided by UltraBotanica (Oklahoma, USA). All analytical certificates and product specifications are provided by UltraBotanica. We open the capsule of riboflavin-5-phosphate (100 mg), dissolve it in a beaker with 20 ml of sterile water and spray on the wound area to apply. Spray on the wound and wait 15 minutes. We shone a blue laser (447 nm) and a violet laser (375 nm) into the wound area. The output power for all wavelengths is set at 100 mW. The energy density was set at 10 joules per square centimeter for the wound area and was set at 2 joules per square centimeter around the wound area. The wound surface is irradiated with violet and blue wavelength lasers. Then we applied at least 10 billion of exosomes on a whole wound area. For large scale wounds, we diluted the exosomes in at least 5ml volume.

RESULTS

Figure 1: In the right, the wound is before starting the procedure. In the bottom right, the results are after two weeks. In the left, the results are after the sixth week

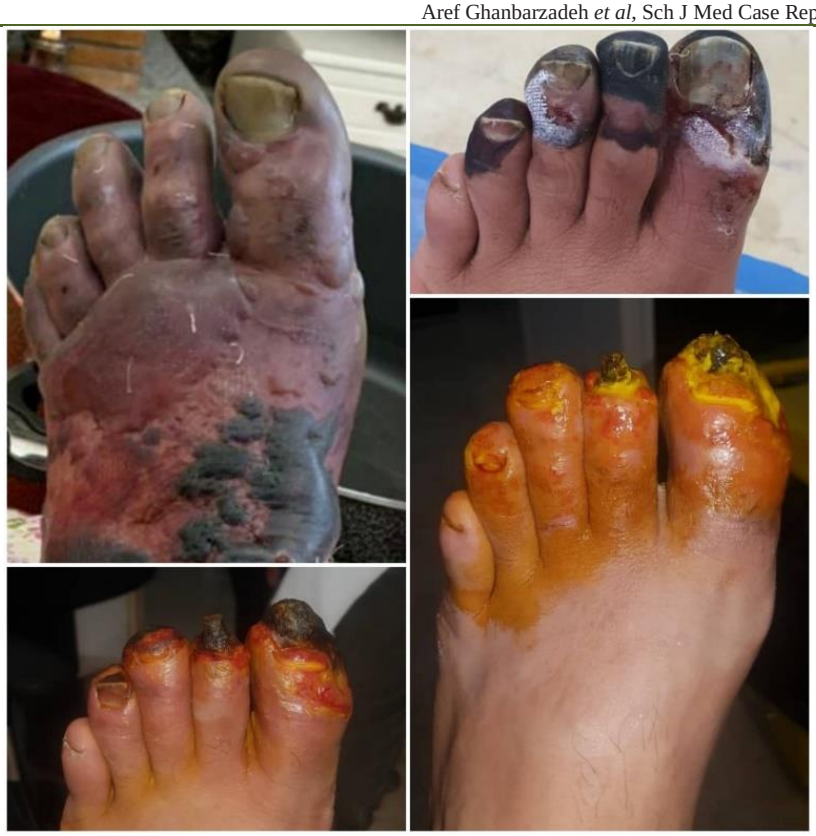

Figure 2: In the top left, the injury is before starting the procedure. In the top right, the results are after the sixth week. In the bottom right, the results are after the eighth week, and in the bottom right, the results are after the 10th week

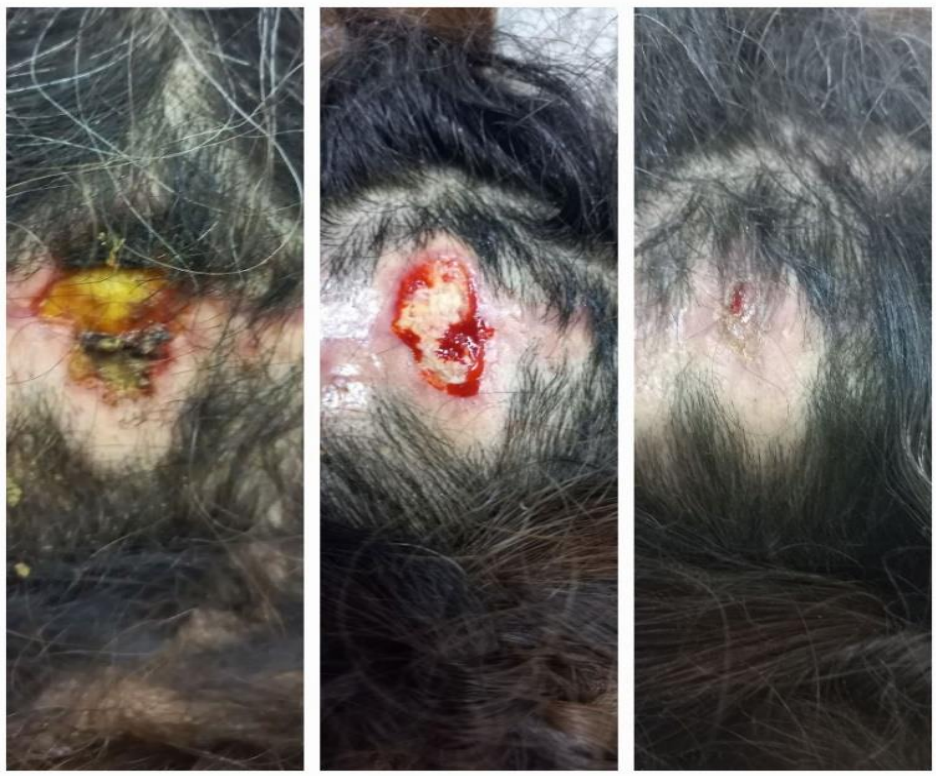

Figure 3: In the left, the wound is before starting the procedure. In the middle, the results are after the third week. In the right, the results are after four weeks

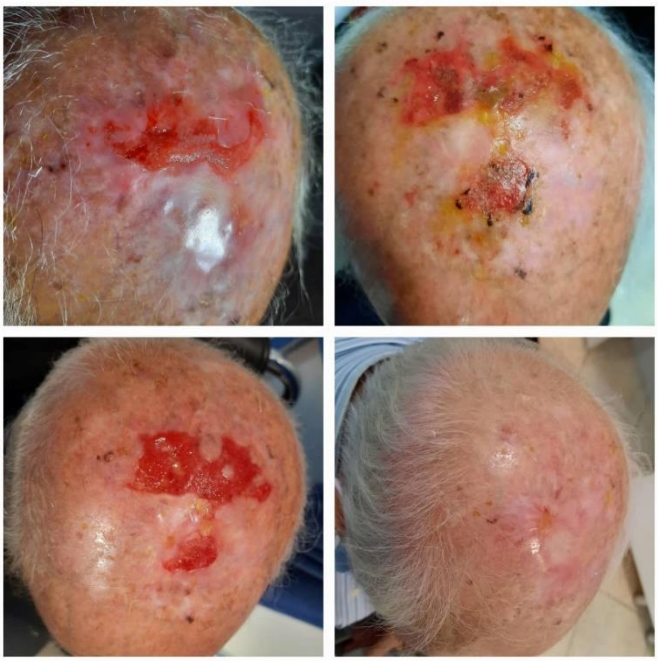

Figure 4: In the top left, the wound is before starting the procedure. In the top right, the results are after the second week. In the bottom left, the results are after the fourth week, and in the bottom right, the results are after the fifth weeks

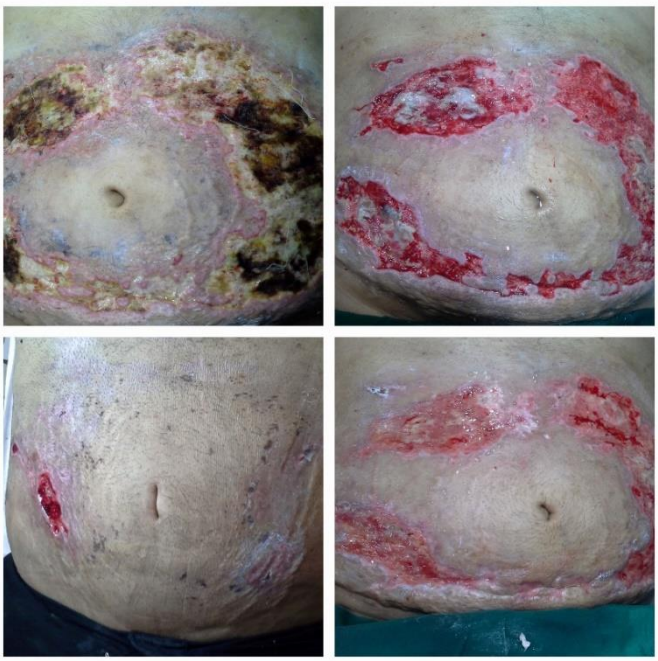

Figure 5: In the top left, the wound is before starting the procedure. In the top right, the results are after the second week. In the bottom left, the results are after the fourth week, and in the bottom right, the results are after the seventh week

CONCLUSION

Riboflavin PDT accelerates wound closure in severe wounds. Topical riboflavin PDT stimulates wound healing by enhancing epithelial regeneration, promoting angiogenesis, and regulating skin homeostasis. This work provides a preliminary theoretical basis for the clinical use of ALA-induced topical PDT in skin wound healing. Low doses of riboflavin PDT activate cell proliferation, angiogenesis and regulate skin homeostasis, promoting wound healing by activating local production of ROS. Our results suggest that topical low-dose riboflavin PDT can be used clinically for the healing of severe and large-scale wounds and injuries.

Increasing evidence of the therapeutic potential of MSC-derived exosomes in promoting skin wound healing seems promising. Exosomes derived from human umbilical cord or adipose MSCs enhance the proliferation and migration of skin cells. Exosomes have been shown to play an important role in skin wound healing. Exosomes contain microRNAs and participate in intracellular communication. We revealed that exosomes secreted by MSCs contain miR-223, which contributes to macrophage polarization. miR-223, which suppresses classical inflammatory pathways and enhances alternative anti-inflammatory responses, is a novel regulator of macrophage polarization and may utilize multiple mechanisms to promote healing. skin wounds. Regardless, we think that through the antiseptic mechanism of PDT combined with the regenerative effects of exosome therapy, can achieve better results in clinics looking at drug-resistant wounds on a large scale.

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