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Therapeutic Effects of Human Mesenchymal Stem Cell–Derived Exosomes on Wound Healing in a Diabetic Mouse Model

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Abstract: Diabetes mellitus (DM) affects over 537 million adults worldwide and is associated with a wide range of complications, among which chronic diabetic wounds—particularly diabetic foot ulcers (DFUs)—pose a serious clinical challenge. These wounds are notoriously resistant to standard therapies due to persistent hyperglycemia, impaired cellular function, chronic inflammation, and defective tissue remodeling. This study investigates the therapeutic potential of human mesenchymal stem cell–derived exosomes (MSC-Exos) for promoting wound healing in a diabetic mouse model, aiming to provide a cell-free, regenerative treatment alternative for chronic diabetic wounds. Full-thickness circular skin wounds were created on the dorsal surface, and mice were treated with MSC-Exos, recombinant human epidermal growth factor (rhEGF), or a negative control. MSC-Exos treatment resulted in significantly faster wound closure, averaging 22 days, compared to 27–30 days in the rhEGF group and negative control group. Histological analysis demonstrated reduced inflammation and more complete skin regeneration, including the development of hair follicles and sebaceous glands. These findings indicate that MSC-derived exosomes effectively enhance diabetic wound healing and offer potential as a novel therapy for chronic wounds.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder primarily characterized by persistent hyperglycemia with a global prevalence of approximately 10.5%, affecting over 537 million adults worldwide, making it one of the most pressing global health challenges[1]. Its pathophysiology involves absolute or relative insulin deficiency and/or decreased peripheral insulin sensitivity[2]. As a multifactorial disease, diabetes arises from the interplay between genetic predisposition and environmental risk factors such as obesity and sedentary behavior[3, 4]. Poor long-term glycemic

control can lead to progressive damage across multiple organ systems, resulting in serious complications such as microvascular damage (retinopathy, nephropathy, neuropathy)[5], macrovascular disease (heart attack, stroke, peripheral artery disease)[6], poor wound healing[7], increased infection risk[8], and cognitive decline[9].

Among all these complications, chronic diabetic wounds, such as diabetic foot ulcers (DFUs), have become a major clinical challenge, often resistant to conventional therapies[10]. Poor wound healing is driven by persistent hyperglycemia, leading to complex molecular, cellular and tissue disruptions[11, 12]. At the molecular level, advanced glycation end-products (AGEs) accumulate and activate the receptor for AGEs (RAGE), triggering NF-κB-mediated inflammation[12, 13]. At the cellular level, essential repair cells such as keratinocytes and fibroblasts exhibit reduced function[12]. On the tissue level, collagen deposition is abnormal, epithelialization is delayed, and chronic inflammation persists[14]. Clinically, diabetic wounds may begin to heal similarly to normal wounds but exhibit significant delays during the proliferation and remodeling phases[15]. This biphasic delay highlights the need for therapies targeting the later stages of wound healing. Epidemiological studies suggest that 15–25% of diabetic patients develop chronic ulcers, and around 20% of those require amputation[10]. Despite the large patient population and the severity of diabetic foot ulcers, current treatment options remain limited and often inadequate. Standard care—including wound debridement, infection control, pressure offloading, and topical dressings—primarily focuses on symptom management and fails to address the underlying biological impairments associated with diabetic wound healing[16]. As a result, many patients continue to experience delayed healing, recurrent ulcers, and even limb amputation, underscoring the urgent need for more effective and targeted therapeutic strategies[16].

Stem cell therapy has emerged as a cornerstone in regenerative medicine and the treatment of chronic diseases. Among various stem cell types, mesenchymal stem cells (MSCs) are the most extensively studied due to their capacity for tissue regeneration and immune modulation[17]. MSCs can be derived from several sources, including bone marrow, adipose tissue, and umbilical cords[17]. Since their first application in Type 1 diabetes treatment in 2007, MSCs have demonstrated the ability to stabilize blood glucose levels[18, 19]. Subsequent clinical trials in Type 2 diabetes patients have reported beneficial outcomes such as reduced fasting and postprandial glucose levels, enhanced insulin sensitivity, increased C-peptide production, and lowered HbA1c levels[19].

A promising alternative to cell-based therapies is the use of exosomes (Exos)—nano-sized extracellular vesicles secreted by cells. MSC-derived exosomes (MSC-Exos), typically 30–150 nm in diameter, retain many of the therapeutic properties of their parent cells[20]. MSC-Exos, secreted via the endosomal pathway, are lipid bilayer vesicles enriched in cytokines, growth factors, and miRNAs that modulate recipient cell function[21]. Compared to whole-cell therapy, MSC-Exos offer distinct advantages, including greater stability, reduced immunogenicity, ease of storage and transport, and more controlled dosing[22]. They have shown regenerative, anti-inflammatory, angiogenic, and immunomodulatory properties in various preclinical models[23], including those involving cardiovascular[24], neural[25], and musculoskeletal disorders[26]. For example, studies have confirmed that bone marrow–derived MSC-Exos improve pancreatic β-cell function in diabetic models, underscoring their potential in metabolic disease treatment[27].

Given the limitations of current diabetic wound therapies, this study investigates the therapeutic efficacy of human MSC-Exos in treating chronic wounds in diabetic mice. The goal is to evaluate their regenerative and anti-inflammatory effects and assess their translational potential as a novel

treatment strategy for diabetic wound management.

2. Materials and Methods

2.1 Main Instruments

The main instruments employed in this study included ultracentrifuge (Thermo Fisher Scientific, Waltham, MA, USA), Animal anesthesia machine (RWD Life Science, Shenzhen, China), paraffin microtome (Leica Microsystems, Wetzlar, Germany), and polarized light photomicrography microscope (Nikon Precision, Otawara, Japan).

2.2 Chemicals

Isoflurane was obtained from RWD Life Science Co., Ltd. (Shenzhen, China). Beifu Xin (rh-bFGF gel) was purchased from Essex Bio-Pharmaceutical Co., Ltd. (Zhuhai, China), Carbomer was purchased from Innochem Technology Co., Ltd. (Beijing, China), H&E staining solution, Sirius red staining solution, Xylene and Neutral balsam were purchased from Beyotime Biotechnology (Shanghai, China).

2.3 Experimental Animals

Male ob/ob diabetic mouse models were procured from Cyagen Biosciences Co., Ltd., based in Suzhou, China. All tested mice were housed under standard laboratory conditions with suitable room temperature and relative humidity.

2.4 Isolation of MSC-Derived Exosomes

Mesenchymal stem cell culture supernatant was collected into 50 mL centrifuge tubes and subjected to sequential centrifugation at 4 ℃: 300 g for 5 min to remove dead cells, 2,000 g for 10 min to remove cell debris, 10,000 g for 20 min to remove microvesicles. The clarified supernatant was then passed through a 0.22 μm filter and ultracentrifuged at 100,000 g for 70 min to pellet exosomes. The pellet was washed once or twice with PBS to remove contaminating proteins and finally resuspended in PBS for storage.

2.5 Preparation of Exosome-Loaded Gel

An appropriate amount of carbomer powder was mixed into 1mL PBS until fully hydrated to form a uniform hydrogel. Five aliquots of 100 μL gel were left untreated (gel group), and five aliquots of 90 μL gel were each mixed with 10 μL exosome suspension (containing 1×10^9 exosomes) to yield 100 μL exosome-loaded gel.

2.6 Establishment of Full-Thickness Skin Defect Model in Diabetic Mice

Male ob/ob mice were anesthetized with isoflurane. After hair removal with depilatory cream, an 8 cm–diameter circular area was marked on the dorsal skin, and full-thickness skin was excised with scissors to create an 8 cm wound.

2.7 Grouping and Treatment of Wound Model

Immediately after model establishment, mice (n = 5 per group) were randomized into negative control: no treatment, positive control: daily topical application of recombinant bFGF gel, exosome group: daily topical application of 1×10^9 exosomes in carbomer gel and gel group: daily topical application of carbomer gel alone.

2.8 Wound Healing Assessment

Wounds were photographed on days 0, 3, 6, 10, 13, 17, 20, 24, 27, and 31. Wound area was measured in Image J, and healing rate was calculated as:

$$\text{Healing Rate} = (\text{Initial Area} - \text{Unhealed Area}) / \text{Initial Area} \times 100\%$$

2.9 Hematoxylin & Eosin (H&E) Staining

At the study endpoint, mice were anesthetized and wound tissues were excised and fixed in 4% paraformaldehyde for 48 hours. The samples were then dehydrated through a graded ethanol series (50% to 100%), embedded in paraffin, and sectioned at a thickness of 5 μm. Tissue sections were deparaffinized in xylene, rehydrated through descending ethanol concentrations to water, stained with hematoxylin for 5-10 minutes, differentiated in acid alcohol, rinsed, and subsequently stained with eosin for 3 minutes. Following staining, sections were dehydrated, cleared in xylene, and coverslipped with neutral balsam. Under microscopy, nuclei appeared blue, while the cytoplasm stained pink.

3. Results and Discussion

3.1 Wound Closure over Time

Photographic documentation (Figure 1) captured the dynamic progression of wound healing across all experimental groups throughout the study period. At early time points, all groups exhibited a mild initial enlargement of the wound area, likely due to inflammation, tissue edema, and mechanical stress associated with the wound environment. This was followed by a gradual contraction of the wound margins, indicating the initiation of the healing process.

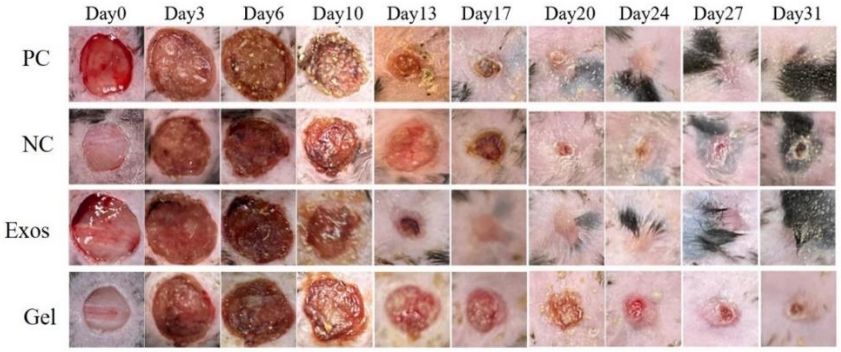

Figure 1: Wound healing progression over 31 days in ob/ob diabetic mouse model.

To complement the visual observations and enhance interpretability, simulated wound images were generated using Image J software (Figure 2). These overlays allowed for a more intuitive and

quantitative visualization of wound closure dynamics over time. As demonstrated in both the photographic and simulated images, by day 13, substantial wound contraction was evident in the exosome (Exos) and positive control (PC) groups, reflecting accelerated healing activity. In contrast, the negative control (NC) and gel-treated (Gel) groups showed minimal wound size reduction at this stage, indicating delayed or suboptimal healing responses.

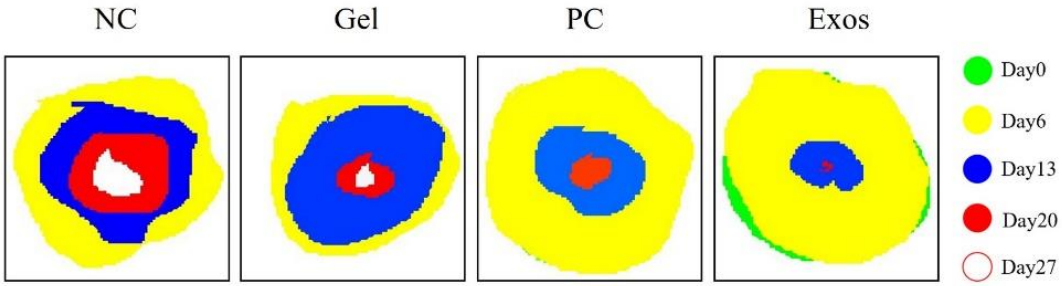

Figure 2: Simulated image of wound area changes over time in diabetic mice.

By day 20, wounds in the Exos group were nearly closed, with only small residual gaps remaining at the wound margins. By day 27, both the Exos and PC groups had achieved complete wound closure, demonstrating their effectiveness in promoting rapid and robust tissue repair. However, the Gel and NC groups still exhibited incomplete healing, with clearly visible unhealed wound areas.

By the final observation point on day 31, wounds in the Exos and PC groups were not only fully closed but also showed signs of advanced tissue regeneration, including re-epithelialization and visible hair regrowth, suggesting restoration of skin function and architecture. In contrast, the wounds in the NC and Gel groups remained partially open, with no evidence of complete epidermal restoration or appendage regeneration.

In summary, the exosome-treated group demonstrated the most efficient and accelerated wound healing, achieving rapid closure and superior tissue regeneration. The PC group also exhibited strong healing performance, though slightly slower than the exosome group. The gel-treated group showed modest improvement, with a healing trajectory lagging behind the exosome and positive control groups. The NC group, receiving no active treatment, exhibited the slowest and least effective wound healing, highlighting the therapeutic advantages of exosome-based intervention.

3.2 Quantification of the Healing Rate and Average Healing Time

To quantitatively evaluate the wound healing dynamics and substantiate the photographic findings, wound closure was assessed over time and expressed as healing rates (Figure 3). These data reveal significant differences in healing trajectories among the experimental groups. In the early phase (Day 3 to Day 10), the Exos group exhibited a moderate advantage, likely reflecting its effectiveness in initiating early tissue repair. By Day 13, the Exos group displayed a further acceleration in healing rate, maintaining the most rapid and consistent progression toward full wound closure. Although the PC group did not show any advantage in the early stages, the wound healing speed significantly accelerated after 13 days.

From Day 13 onward, both the Exos and PC groups demonstrated significantly improved healing compared to the Gel and NC groups, ultimately achieving near-complete closure (~98%) by Day 24. The Gel group exhibited a slower and less consistent healing trajectory, while the NC group showed minimal improvement throughout the study. These trends underscore the superior regenerative

efficacy of exosome-based treatment, likely driven by their anti-inflammatory, angiogenic, and pro-reparative bioactivity.

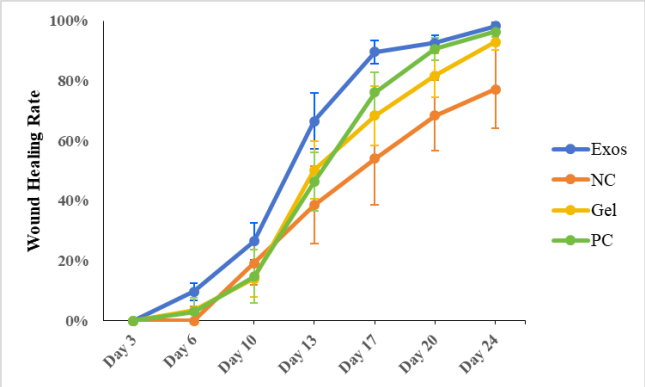

Figure 3: Healing rate curves over time in different groups of mice.

To further quantify treatment efficacy, the total wound healing time—defined as the time required for complete re-epithelialization—was also calculated and is shown in Figure 4. Consistent with the wound healing rate analysis, the Exos group achieved the shortest healing time, with wounds resolving significantly earlier than those in the Gel and NC groups. The PC group also exhibited a reduced healing time compared to Gel and NC, although to a lesser extent than the Exos group. The Gel and NC groups displayed the longest healing durations, further reinforcing the suboptimal performance of these treatments.

Taken together, the temporal healing profiles (Figure 3) and endpoint healing times (Figure 4) provide strong and complementary evidence for the effectiveness of exosome therapy. The Exos group not only accelerated the rate of wound contraction but also minimized the overall time required for complete tissue regeneration. These findings highlight the potential of exosome-based interventions as a highly effective strategy for enhancing wound healing outcomes.

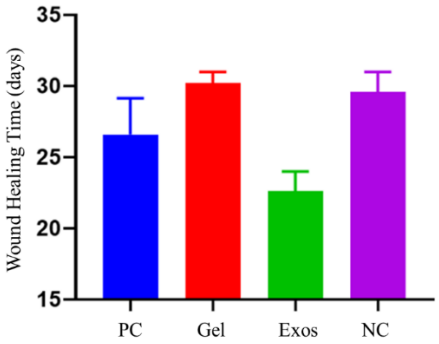

Figure 4: Average wound healing time in different groups of diabetic mice.

3.3 Histopathological Analysis

Hematoxylin and eosin (H&E) staining at the study endpoint (Figure 5) provided histological insights into the degree of tissue regeneration and inflammation in each treatment group. In the negative control and gel groups, the skin tissue exhibited severe inflammatory cell infiltration, characterized by dense accumulations of immune cells within the dermis and subcutaneous layers. Additionally, there was evidence of disrupted and disorganized skin architecture, including an

incomplete or absent epidermal layer, irregular collagen deposition, and a lack of structural features such as hair follicles and sebaceous glands, indicating poor wound healing and ongoing inflammation.

In contrast, the exosome and positive control groups demonstrated well-organized epidermal and dermal layers, with clear evidence of re-epithelialization and tissue regeneration. In these groups, the inflammatory response was markedly reduced, and the skin structure closely resembled that of normal, healthy tissue. Notably, hair follicles and sebaceous glands were observed within the regenerated dermis, suggesting a more complete restoration of skin appendages and improved functional recovery. These findings corroborate the superior therapeutic effects of the exosome and positive control treatments in promoting not only wound closure but also high-quality skin regeneration.

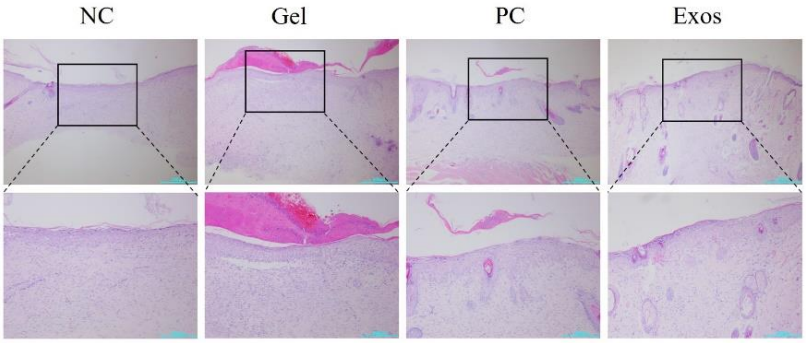

Figure 5: H&E staining images at the experimental endpoint for each group.

4. Conclusions

In the present study, we demonstrated that topical application of 1×10^9 MSC-Exos formulated in a carbomer gel led to significantly accelerated wound healing in diabetic ob/ob mice. Compared to untreated controls, which required approximately 30 days for full closure, the exosome-treated group achieved complete re-epithelialization within 22 days—representing a marked improvement in healing kinetics. In addition to macroscopic closure, histological analysis at the study endpoint revealed well-organized tissue structure, re-formation of skin appendages (including hair follicles and sebaceous glands), and marked reduction in inflammatory infiltration in the exosome group, indicating not only faster but also higher-quality tissue regeneration.

These results are consistent with and reinforce previous studies that have shown MSC-Exos can modulate wound healing by delivering paracrine signals such as microRNAs, cytokines, and angiogenic factors, which in turn influence the behavior of keratinocytes, fibroblasts, and immune cells. The exosomes used in this study were isolated using ultracentrifugation, the gold-standard method for exosome purification, ensuring high purity and biological functionality. Quantitative wound assessment was performed using ImageJ-based analysis, providing objective and reproducible data to support the therapeutic efficacy of the formulation.

In conclusion, our findings provide strong preclinical evidence supporting the use of topical MSC-Exos as a safe, effective, and scalable therapy for diabetic wound management. Given their cell-free nature, low immunogenicity, and ease of storage and handling, exosome-based treatments hold significant translational potential. Future studies should focus on mechanistic investigations, dose optimization, and clinical validation to accelerate the development of exosome-based therapies for widespread clinical use in chronic wound care.

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