Minireview Article

MACROSCOPIC WOUND HEALING IN THE LIMBS OF DIABETIC RATS TREATED WITH AUTOLOGOUS MESENCHYMAL STEM CELL MICROGRAFTS

ABSTRACT

Mesenchymal stem cells (MSCs), undifferentiated cells with high proliferative potential, are present in small quantities in secretions and various adult tissues. The wide distribution of these cells and the absence of ethical concerns regarding their use allow the extraction of MSCs from tissue fragments using the Rigenera® technology, which enables the production of micrografts. Due to their reparative and paracrine potential, MSCs have emerged as an alternative to standard pharmaceutical treatment modalities for wound healing. The present study aimed to analyze the macroscopic performance of MSCs in wound healing in diabetic rats, considering the pathological characteristics and the dysregulation of the inflammatory response that complicates healing. Wistar rats were used, divided into four groups: control (CT), control treated with micrografts (CTST), diabetic (DB), and diabetic treated with micrografts (DBST). Lesions approximately 1 cm² in size were made on the hind paw, affecting the skin, subcutaneous tissue, and muscle. In the treated groups, autologous micrografts obtained via the Rigenera® system were used. Macroscopic parameters such as hyperemia, secretion, granulation tissue formation, and epithelialization were analyzed. The DBST group showed significant advances in tissue reconstruction, with complete tissue recomposition observed after 14 days. Thus, the Rigenera® system proved to be efficient in obtaining autologous micrografts for tissue regeneration in lesions of diabetic rats.

Keywords: Diabetes 1; Mesenchymal stem cells 2; Tissue injury 3; Tissue regeneration 4; Wound healing 5.

1. INTRODUCTION

The rapid global increase in diabetes mellitus (DM) has reached alarming levels, necessitating special attention. This chronic disease is characterized by elevated blood glucose levels (hyperglycemia). In type I diabetes mellitus (DM I), hyperglycemia results from insufficient insulin production due to an autoimmune process. In type II diabetes mellitus (DM II), however, hyperglycemia arises from reduced insulin secretion by the pancreas or cellular resistance to insulin in glucose metabolism. According to the World Health Organization, approximately 422 million individuals globally are affected by DM, with a significant increase in incidence observed in recent decades. It is estimated that DM directly contributes to 1.5 million deaths annually. Studies indicate that severe pathological damage to the vascular system in DM patients, resulting from abnormal glucose metabolism and other metabolic disturbances, is well-documented in clinical practice [1].

Diabetic foot is one of the most common complications of diabetes mellitus and is a leading cause of hospitalization among diabetic patients. Lower limb issues such as diabetic neuropathy, peripheral arterial disease, ulcerations, and amputations affect the diabetic population at twice the rate observed in non-diabetics, particularly among individuals aged 40 and older [2; 3]. Moreover, in developing or emerging countries, social and behavioral factors further exacerbate the risk of foot disease. Such factors include walking barefoot, lack of proper information, delayed medical consultation, and a shortage of available specialists [4].

It is estimated that at least 15% of diabetic patients will develop a foot injury during their lifetime [2]. The wound healing process generally involves phases such as coagulation and hemostasis, inflammation, proliferation, and tissue remodeling (re-epithelialization and contraction). In diabetic patients, however, wounds often take considerably longer to heal as they frequently fail to progress from the inflammatory phase to the resolving stages of proliferation and remodeling. This delay is attributed to reduced vascular perfusion, thickening of the extracellular basal membrane, and dysregulation of collagen synthesis [5; 6]. This impaired healing process can lead to secondary conditions such as depression and family isolation, as well as emotional distress [3]. Additionally, due to the high prevalence of these wounds and the complexity of treatment, particularly in lower limbs, there is a significant decrease in patient productivity and a notable increase in healthcare costs, especially in cases resulting in amputation [7].

Preventive education is critical for effective management, aiming to prevent wound development by disseminating general health care knowledge (such as glycemic control) and specific care (such as podiatric attention). This strategy is considered among the most effective, simple, and feasible [8]. In cases where the condition is established, prompt and effective treatment of diabetic foot wounds requires a comprehensive approach, encompassing classification systems to accurately diagnose the condition and its specificities, assessment of risk factors, selection of the most appropriate antimicrobial therapy, and potential surgical interventions [7; 9].

The most effective treatment protocol for diabetic foot ulcers includes wound debridement, infection control, revascularization promotion when necessary, and pressure relief on the ulcer [10; 11]. Adjunctive methods have also been suggested with potential benefits in the complex healing process, such as hyperbaric oxygen therapy, negative pressure wound therapy, and the use of specific growth factors [12]. However, these therapies present limitations and reservations, primarily due to the need for further studies to robustly support their efficacy and verify possible adverse effects [11; 13; 14]. Consequently, the effects of DM, coupled with infections and vascular injuries, continue to impede a sustained healing process, often resulting in chronic lesions [15].

In this context, mesenchymal stem cells (MSCs) emerge as a promising therapeutic alternative to accelerate wound recovery and prevent amputations. These cells are classified as multipotent, non-hematopoietic adult stem cells, with the ability to self-renew and differentiate into various cell types, including mesodermal, ectodermal, and endodermal lineages [16; 17]. MSCs can be isolated from several sources, including adipose tissue, bone marrow, and neonatal tissues, and must express basic markers such as CD73, CD105, and CD90, while lacking CD34, CD14, CD45, CD11b, CD19, and HLA-II [18; 19]. Moreover, these cells have been shown to migrate to injured areas and interact with the local microenvironment, secreting biomolecules involved in processes such as fibrosis, proliferation, apoptosis, chemotaxis, immunomodulation, and angiogenesis. These effects allow MSCs to promote the regeneration of damaged tissue, strengthen the immune response, and prevent cellular apoptosis [6; 20; 21].

However, the cultivation of stem cells for clinical application requires high standards of quality and quantity. Traditional methods of expanding these cells involve two-dimensional culture techniques, xenogeneic media, and enzymatic chemical solutions, which limit cell development and may lead to loss of clonal capacity and differentiation potential. Additionally, these methods demand significant time and financial resources [22; 23].

An innovative technology is currently being applied in regenerative medicine due to its capacity to efficiently obtain viable mesenchymal stem cells (MSCs). The Rigenera® System employs a biological tissue disaggregator, known as Rigeneracon®, to extract 50µm micrografts from a small sample of connective tissue from the individual undergoing intervention [24; 25]. This technique enables the minimally invasive extraction of autologous micrografts, ready for immediate use, without the need for traditional cell culture [26; 27].

Several scientific studies have reported positive outcomes with the use of the Rigenera® System across different pathological conditions, including surgical wound dehiscence, bone lesions, myocardial function loss, cartilage diseases, hard-to-heal wounds, dermal tissue loss, and androgenetic alopecia [26; 27; 28; 29; 30; 31; 32].

Accordingly, the present study aims to investigate the efficacy of autologous micrografts containing MSCs in wound healing in diabetic rats. This clinical model was selected due to the difficulty diabetic patients experience in completing the healing process, especially in wounds located on the extremities.

2. METHODOLOGY

Animal handling adhered to the international principles for biomedical research involving animals as stipulated by the International Guiding Principles for Biomedical Research Involving Animals [33]. The guidelines of the Brazilian Directive for the Care and Use of Animals for Scientific and Educational Purposes (2013) were also strictly followed in conducting this project. Fifty clinically healthy Wistar rats of both sexes, weighing between 200 and 300 g and aged 90 days, were used, provided by the Animal Facility of the Department of Animal Morphology and Physiology at the Federal Rural University of Pernambuco. The animals were housed in polycarbonate cages (41x34x16 cm) lined with pinewood shavings, which were changed every two days. The environment was controlled with a temperature of 22ºC ± 2ºC, a 12-hour light cycle (400 Lux), and a 12-hour dark cycle, with free access to food and water.

The animals were divided into four groups: control (CT), control treated with micrograft (CTST), diabetic (DB), and diabetic treated with micrograft (DBST). At 90 days of age, the rats in the diabetic group received an intraperitoneal injection of streptozotocin (60 mg/kg of body weight) dissolved in 0.1 M citrate buffer at pH 4.5. The control group animals underwent the same procedure, receiving only the citrate buffer. Blood glucose levels in the diabetic groups were measured 8 and 72 hours after the streptozotocin administration using glucose test strips; only animals with glucose levels above 200 mg/dL were included in the diabetic groups. During the treatment period, postprandial glucose levels were monitored to ensure the persistence of the diabetic condition.

Wounds were created using a sterile scalpel, with incisions reaching the skin, subcutaneous tissue, and muscle tissue. All animals were anesthetized with ketamine and xylazine at manufacturer-recommended dosages for the surgical procedure. The wounds, approximately 1 cm² in rectangular shape, were made on the hind paw of the left antimere.

Micrografts were obtained by performing trichotomy and collecting a 1 cm² dermal sample from the right lateral side of the animals' spinal region. The collected material was

fragmented into smaller pieces and processed in the Rigenera® System with 1 mL of saline solution, followed by disaggregation for 5 minutes. The collection area was sutured, and the resulting micrografts were applied to the wounds on the donor animal's own paw. The micrografts were administered using a 30G x 6 mm hypodermic needle inserted at a 45-degree angle, infiltrating the micrografts into the four corners of the wound.

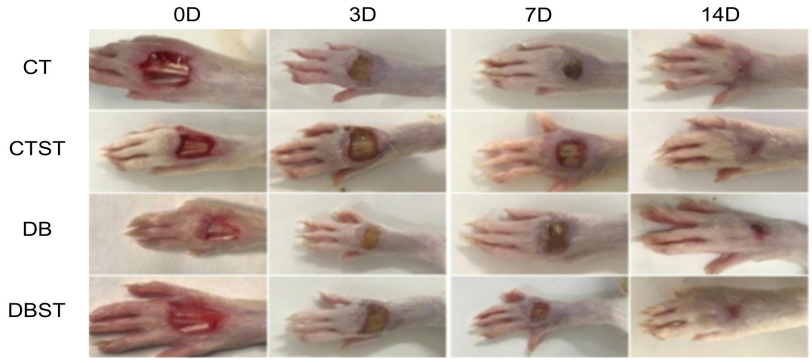
Macroscopic evaluations of the wounds were conducted on days 0, 3, 7, and 14 following autologous micrograft treatment, with photographs taken on these occasions. The analyzed parameters included the presence or absence of hyperemia, secretion, granulation tissue formation, and the degree of epithelialization (wound closure). Healing outcomes were compared across the experimental groups.

3. RESULTS AND DISCUSSION

The observations indicated improved wound healing in animals subjected to cell therapy. In the DBST group, the rate of tissue reconstruction was similar to the other groups up to the third day of healing. However, from the seventh to the fourteenth day, the progress was significantly greater, with complete tissue recomposition in the injured area. This result may be attributed to the self-renewing, proliferative, and, most importantly, paracrine properties of mesenchymal stem cells (MSCs).

José et al. [34] found that the characteristics of MSCs from diabetic and non-diabetic rats, tested in vitro, were comparable in terms of morphology, growth rate, mesenchymal profile, and differentiation into standard lineages. Clinically, these authors also reported that both cell groups achieved satisfactory results in controlling blood glucose levels, reducing body weight, and improving cardiac electrical remodeling in experimental diabetic models. Although some genetic differences have been reported in the literature, MSCs from donors with type I diabetes mellitus (DM I) retain phenotypic and functional similarity with those from healthy individuals, particularly in their immunomodulatory potential and homing ability. This reaffirms their viability for autologous therapies [35; 36].

Figure 1 – Visual Comparison Between Groups. CT: Control Group; CTST: Control Group with Treatment; DB: Diabetic Group; DBST: Diabetic Group with Treatment.



Source: Authors (2024).

Tissue healing begins with the release of alpha granules by platelets, which recruit immune cells to prevent infection and remove necrotic tissue. This is followed by angiogenesis, granulation tissue formation, and re-epithelialization via cell migration, proliferation, and maturation. Later, these processes diminish, and the collagen in the extracellular matrix undergoes remodeling. Within this healing process, mesenchymal stem cells (MSCs) play a critical role in mediating epithelial-mesenchymal interactions, which are essential for skin integrity [6].

Regarding secretion, no exudate was observed in any evaluated groups. However, starting on the third day, erythema was detected exclusively in the DB group, in contrast to the other groups. This difference may be attributed to the MSCs present in the micrografts, which, through secreted factors, promote immunomodulation by shifting macrophage polarization from the M1 to the M2 phenotype. M2 macrophages are known for their healing and resolution properties, induced by anti-inflammatory cytokines such as IL-4 and IL-10 [37]. A study on micrografts obtained via the Rigenera® system reported high levels of MSC markers CD73 and CD90, absence of MHC class II expression, and positive expression of the IL-10 gene—all associated with anti-inflammatory activities [38].

There is mounting evidence that the bioactive secretome released by MSCs, including DNA, RNA, microRNA (miRNA), and proteins, may possess equal or even greater tissue repair capacity than MSCs themselves. A study administering MSC-derived secretome (exosomes) from human umbilical cord cells to diabetic rats with streptozotocin-induced wounds showed a marked acceleration in wound closure, supporting the results observed in this study [36]

All groups exhibited similar granulation tissue formation. However, by the seventh day, wounds in the DBST group showed re-epithelialization levels comparable to those in the CT and CTST groups, unlike those in the DB group. This observation may be explained by the paracrine activity of MSCs, which secrete growth factors such as FGF and VEGF-A, stimulating revascularization and promoting the proliferation, migration, and differentiation of endothelial cells in blood vessels [39; 40]. The DBST group demonstrated remarkable tissue regeneration rates, achieving complete tissue restoration through angiogenesis, which occurs during the proliferative phase of wound healing. The necessary perfusion for fibroblasts—responsible for producing granulation tissue—is directly associated with the angiogenic potential of MSCs [39; 41].

The findings of this study align with other research in the field. Francesco et al. [42] reported that autologous micrografts obtained through the Rigenera® system accelerated tissue repair in chronic ulcers, including diabetic wounds, noting reductions in wound size, increased granulation, and decreased exudate. In vitro tests by the authors confirmed the presence of multipotent MSCs with positive expression of CD73, CD90, and CD105 markers.

Kuo et al. [43] investigated diabetic wound healing and observed significantly elevated levels of EGF, VEGF, prolyl 4-hydroxylase (rPH), and Ki-67 (a cell proliferation marker) in MSC-treated groups compared to the control group. Regeneration and neoangiogenesis were attributed to the paracrine and autocrine activities of MSCs. Similar results were reported by Yan et al. [44], where MSCs promoted endothelial cell differentiation and activated AKT signaling pathways, regulating angiogenesis.

Another critical aspect of wound healing is the role of keratinocytes, essential cells in the epidermis involved in all phases of the healing process. MSCs have demonstrated the ability to promote tissue regeneration not only through the mechanisms mentioned but also by forming new keratinocytes and maintaining their functionality within the local microenvironment [45]. This effect is linked to increased levels of CCL2 and EGF, which are upregulated following stem cell therapies [46].

4. CONCLUSION

The use of autologous micrografts in the treatment of non-healing wounds emerges as a promising therapeutic alternative, distinguished by its speed, ease of harvesting, and cost-effectiveness. The Rigenera® system proved effective in obtaining autologous micrografts for tissue regeneration in diabetic rat lesions. Macroscopically, the application of

micrograftswas satisfactory, resulting in faster healing with improved granulation tissue formation and reepithelialization, along with an absence of secretion across all groups. The reduction of hyperemia in the treated group (DBST) further supports the immunomodulatory action of mesenchymal stem cells.

Thus, the use of autologous micrografts presents a viable and effective option. However, further studies are required to confirm these findings, optimize application protocols, and validate the safety and efficacy of this treatment. With refined protocols, the Rigenera® system may become widely applicable in treating cutaneous lesions in diabetic patients, particularly for wounds that are difficult to heal.

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