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Original Article

Xenogeneic mesenchymal stem cell biocurative improves skin wounds healing in diabetic mice by increasing mast cells and the regenerative profile

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ABSTRACT

Introduction: Diabetes mellitus (DM) is a chronic disease and a major cause of mortality and morbidity worldwide. The hyperglycemia caused by DM induces micro and macrovascular complications that lead, among other consequences, to chronic wounds and amputations. Cell therapy and tissue engineering constitute recent therapeutic alternatives to improve wound healing in diabetic patients. The current study aimed to analyze the effectiveness of biocuratives containing human mesenchymal stem cells (MSCs) associated with a hydrogel matrix in the wound healing process and related inflammatory cell profile in diabetic mice.

Methods: Biocuratives containing MSCs were constructed by 3D bioprinting, and applied to skin wounds on the back of streptozotocin (STZ)-induced type 1 diabetic (T1D) mice. The healing process, after the application of biocuratives with or without MSCs was histologically analyzed. In parallel, genes related to growth factors, mast cells (MC), M1 and M2 macrophage profiles were evaluated by RT-PCR. Macrophages were characterized by flow cytometry, and MC by toluidine blue staining and flow cytometry.

Results: Mice with T1D exhibited fewer skin MC and delayed wound healing when compared to the non-diabetic group. Treatment with the biocuratives containing MSCs accelerated wound healing and improved skin collagen deposition in diabetic mice. Increased TGF-β gene expression and M2 macrophage-related markers were also detected in skin of diabetic mice that received MSCs-containing biocuratives. Finally, MSCs upregulated IL-33 gene expression and augmented the number of MC in the skin of diabetic mice.

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List of abbreviations

ALT	Alanine transaminase
Arg-1	Arginase- 1
AST	Aspartate transaminase
CD117	Cluster of differentiation 117
CD11c	Cluster of differentiation 11 c
CD206	Cluster of differentiation 206
CD45	Cluster of differentiation 45
DM	Diabetes mellitus
ELISA	Enzyme-linked immunosorbent assay
FcεRI	High affinity IgE receptor
H/E	Hematoxylin/eosin
i.p.	Intraperitoneally
IFN-γ	Interferon γ
IGF-1	Insulin-like growth factor 1
IL-10	Interleukin-10
IL-1β	Interleukin-1 beta
IL-33	Interleukin-33
IL-4	Interleukin −4
IL-6	Interleukin-6
iNOS	Inducible nitric oxide synthase
MC	Mast cell
mMCPT4	Mouse mast cell protease 4
MRC-1	Mannose receptor C-type 1
MSCs	Mesenchymal stem cells
PDGF	Platelet-derived growth factor
RT-PCR	Real time-polymerase chain reaction
STZ	Streptozotocin
T1D	Type 1 diabetes
TGF-β	Transforming growth factor beta
TNF-α	Tumor necrosis factor alpha
VEGF	Vascular endothelial growth factor

Conclusion: These results reveal the therapeutic potential of biocuratives containing MSCs in the healing of skin wounds in diabetic mice, providing a scientific base for future treatments in diabetic patients.

1. Introduction

Type 1 diabetes (T1D) is a metabolic and autoimmune disease characterized by chronic hyperglycemia, which changes blood vessel homeostasis, leading to cardiac and ophthalmic complications, renal failure, neuropathy, and loss of wound healing ability [1,2]. Hemostatic changes resulting from hyperglycemia lead to dysfunction of the healing mechanisms [3]. As a result, wounds on the feet of diabetic people cause about 85% of lower-limb amputations in these patients [4–6]. The normal healing process consists of three main phases: inflammation, proliferation, and remodeling. Several cell types and signaling pathways define the sequence of events [7]. The chronicity of diabetic wounds is mainly due to the lack of resolution of the inflammatory stage and the inability to progress to the proliferative and remodeling stages. Diabetic patients exhibit increased production of interleukins (IL), such IL-1β, IL-6, and tumor necrosis factor-α (TNF-α) in the inflammatory phase of wound healing. On the other hand, they show decreased expression of cytokines associated with inflammation resolution, such as IL-10 and transforming growth factor-beta (TGF-β). The predominance of the pro-inflammatory phenotype and the increased number of neutrophils, which secrete high levels of pro-inflammatory cytokines, cause tissue damage in these patients [8–12]. Dysregulation in the expression of collagen matrix metalloproteinases, dysfunction of the vascular endothelium, deficiency in the capacity of angiogenesis, and failure in the migration and proliferation of fibroblasts and keratinocytes, also contribute to chronic inflammation and defective wound healing [3,13–15].

Mast cells (MC) are naturally resident in the skin and secrete cytokines and growth factors in the healing process. During the inflammatory phase, MC degranulate and secrete pro-inflammatory cytokines that drive the migration of neutrophils and monocytes from the circulation to the inflamed tissues. During the proliferative phase, MC secrete anti-inflammatory cytokines that induce proliferation and differentiation of other immune cells. MC secrete IL-33, an “alarmine” that signals cell damage and has an important role in wound healing [16,17]. MC also express the IL-33 receptor, which is formed by the ST2 protein and the IL-1 receptor accessory protein (IL-1RAcP). The IL-33 receptor triggers signs of survival, maturation, activation, and release of vascular endothelial growth factor (VEGF) by MC [18,19]. Despite many studies, the role of MC and IL-33 in wound healing is still not fully elucidated [20,21].

Cell therapy has become more relevant in the healing process with great potential in the treatment of chronic wounds [22,23]. The administration of mesenchymal stem cells (MSCs) from adipose tissue, umbilical cord, and bone marrow at the edges of wounds significantly improves healing in murine models of diabetes and in preclinical studies [22–28]. MSCs are important in the progression of the healing stages, as they migrate to the injury sites, differentiate into different cell types, and secrete immunoregulatory and angiogenic mediators. In diabetic patients, MSCs resolve the inflammation phase of the healing, recognize pro-inflammatory factors such as TNF-α and interferon-γ (IFN-γ), and secrete anti-inflammatory cytokines such as IL-10 and TGF-β, which contribute to the differentiation of M2 macrophages and favor the healing process. MSCs also act in the initiation of the proliferative phase, through the secretion of growth factors, such as VEGF and platelet-derived growth factor (PDGF) that stimulate proliferation of endothelial cells and fibroblasts [25,29–34]. New studies aimed to create biocuratives composed of hydrogel matrices associated with mesenchymal cells have been launched. They seek to maintain MSCs in the lesion's microenvironment to improve their therapeutic functions [22,25,29,35–37]. Considering that a 3D bioprinting interface allows great reproducibility, standardization, and scalability in the manufacture of biocuratives with mesenchymal cells, in the present study we determined the effectiveness of bioprinted hydrogel biocuratives containing mesenchymal cells in the healing of wounds in diabetic mice. The MSCs' immunological mechanisms on skin mast cells and M2 macrophages was also investigated and the role of IL-33 in this process was determined.

2. Methods

2.1. Diabetes model

Male, 8 weeks-old, C57BL/6 mice were intraperitoneally (i.p.) injected with STZ (40 mg/kg, Sigma Aldrich) for five consecutive days. STZ was diluted in a 0.1 M citrate buffer (pH = 4.5) and injections were performed in mice under 6 h (h) of fasting. Citrate buffer was administered to the non-diabetic groups. During a 30-day period, mice were monitored for weight body and blood glucose levels to confirm the induction of T1D. Only mice with blood glucose above 200 mg/dL, measured twice after a 6 h-fasting, were included in the diabetic group and used for the experimental protocols [38].

2.2. Experimental wound model and application of biocuratives

Fifteen days after the T1D induction (Fig. 1A), mice were anesthetized with xylazine (0.6 mg/kg) and ketamine (0.3 mg/kg), trichotomized on the back, and subjected to a wound of 1 cm^2 performed with sterile surgical scissors [39]. Mice were kept in individual boxes housed under specific pathogen-free conditions, with *ad libitum* access to food and water. The housing room was set to a 12 h light/dark cycle with temperature of about 22 °C. The removed skin was used for histology, characterization of leukocyte populations by flow cytometry, gene expression by RT-PCR, and cytokine quantification by ELISA assay. Immediately after the lesion induction, biocuratives with or without MSCs (derived from the human umbilical cord) were applied. The biocuratives were made available by the startup *In Situ Cell Therapy,* located in Supera Parque de Inovação e Tecnologia de Ribeirão Preto, Ribeirão Preto, Brazil. They were made using an alginate hydrogel matrix [40] and bioprinted with 1×10^5 MSCs, which were administered per square centimeter of the lesion. Mice were divided into four groups: 1) non-diabetic mice with biocurative without MSCs (ND + b), 2) non-diabetic mice with biocurative containing MSCs (ND + bMSC), 3) diabetic mice with biocurative without MSCs (T1D + b), and 4) diabetic mice with biocurative containing MSCs (T1D + bMSC). After 15 days of the wound's induction, the mice were euthanized by an overdose of anesthetics, and a 4 cm^2 sample of the healed skin, with the wound at the center, was collected, divided into parts, and properly stored for the different experimental assays.

2.3. Assessment of the toxicity and MSCs permanence in the wound

The biocuratives were applied immediately after the wounds were performed. After the 15-day healing period, potential hepatic toxicity due to the application of MSCs was determined through serum quantification of aspartate transaminase (AST) and alanine transaminase (ALT) enzymes, using the AST/GOT and ALT/GPT Liquiform kits (Labtest, Brazil). To evaluate the permanence of the cells in the biocurative, a bioluminescence assay was carried out. The HEK293T/17 mesenchymal cell line was transfected with the plasmid pMSCV_Luc2_T2A_Pure that contains the luciferase enzyme 2 gene and the Puromycin N-acetyltransferase gene, which confers resistance to the antibiotic puromycin. The cells were added to the biocuratives, which in turn were applied to the wounds in diabetic and non-diabetic mice. On days 1, 2, 4, 7, and 15 after application of the biocuratives, with or without MSCs, 40 μl of D-luciferin (30 mg/mL) were applied (initially i. p. and then topically) to the wound. Bioluminescence readings were performed in an IVIS® equipment from PerkinElmer.

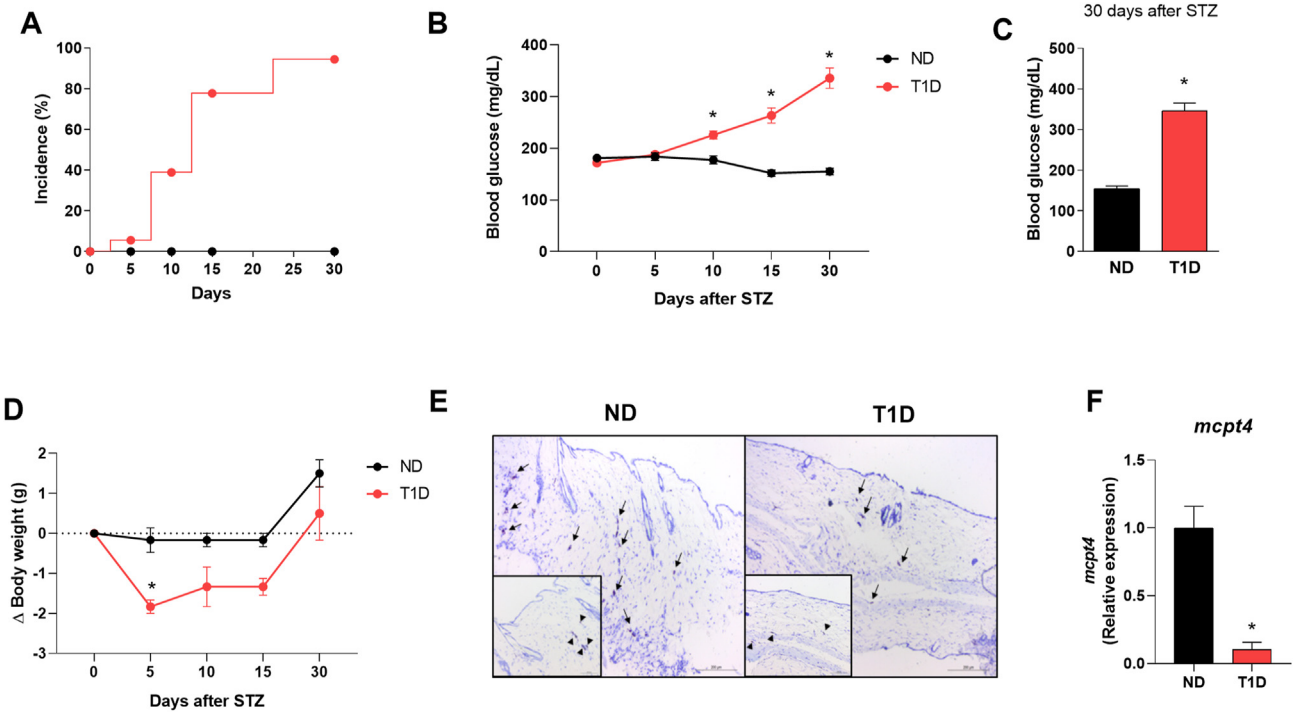

Fig. 1. Clinical parameters and reduction of mast cells in T1D. (A) Incidence of T1D after 30 days of administration of STZ (T1D group) or vehicle [VH (ND group)]. **(B)** Blood glucose kinetics throughout the experimental period. **(C)** Glucose levels 30 days after initiation of STZ administration. **(D)** Body weight variation in diabetic and non-diabetic mice during 30 days. **(E)** Representative photomicrographs of skin stained with toluidine blue for mast cell labeling. Magnification 100x (scale bar: 200 μm) and in insert box 200x (scale bar: 100 μm). **(F)** Mmcpt4 gene expression by RT-PCR. Results are shown as mean ± SEM and are representative of two independent experiments (n=18). Statistical significance was determined by two-way analysis of variance (ANOVA) and Sidak's multiple comparison test **(B, D)** or unpaired t-test **(C, F)**. *T1D *vs* ND; $p < 0,05$. STZ: streptozotocin, T1D: type 1 diabetes, ND: non-diabetic.

2.4. Evaluation of the healing process

After the wound induction and application of biocuratives, the wounds were photographed on days 0, 3, 5, 7, 10 to evaluate the closure. The percentage of healing, calculated by the area of the lesions in the different periods in relation to the time 0, was determined using the ImageJ software.

2.5. Histological analysis of the scarred skin

Histopathological analyzes of the wound region were performed in a quarter of each collected sample. After collection, the skin samples were fixed in paraformaldehyde for 24 h for later inclusion in paraffin. Then, the tissue was sectioned (5 μm), deparaffinized, and stained with Hematoxylin/Eosin (H/E) to analyze the morphology of the recovered tissue. Picrosirius stain, to identify collagen fibers, and Toluidine Blue stain, for the identification of MC, which are stained in violet color, were also performed.

2.6. Analysis of gene expression by real-time PCR (RT-PCR)

Total RNA was extracted from the wound samples using Trizol and the SV Total RNA extraction kit (Promega). The quantitative expression of the genes was analyzed through RT-PCR reactions, using the SYBR Green systems in a Step One Plus equipment (Applied Biosystems – USA). The genes encoding the inducible nitric oxide synthase (iNOS) enzyme and the pro-inflammatory cytokine TNF-α, related to the M1 macrophage phenotype, were analyzed. In relation to the M2 macrophage phenotype, the gene expression of the arginase-1 (Arg-1) enzyme and the mannose receptor C-type 1 (MRC-1) (also known as CD206) was analyzed. In addition, we evaluated the gene expression of the IL-4, and IL-33 cytokines. To determine the presence of MC in the skin, gene expression of mouse mast cell protease 4 (mMCPT4), a marker for connective tissue MC, was quantified. Finally, to evaluate the expression of growth factors, gene expression of TGF-β, VEGF, PDGF, and insulin-like growth factor 1 (IGF-1) was determined. The expression of these genes was normalized by the 18S endogenous gene expression. The results were analyzed based on the value of CT (cycle threshold). The primers used for each gene are listed in Table 1.

Table 1
List of primers used in the RT-PCR.

Target	Primer	Sequence
TNF-α	Forward	5′-TGTGCTCAGAGCTTTCAACAA-3′
	Reverse	3′- AGAGTACGTGGTGGTAGTTC- 5′
iNOS	Forward	5′-CGAAACGCTTCACTTCCAA-3′
	Reverse	3′-TGAGCCTATATTGCTGTGGCT-5′
Arginase-1	Forward	5′- GTTCCCAGATGTACCAGGATTC-3′
	Reverse	3′CGATGTCTTTGGCAGATATGC-5′
MCR-1	Forward	5' - CTCTGTTCAGCTATTGGACGC - 3′
	Reverse	3' - AGTTTAATACAAACCCTCAGGT- 5′
IL-33	Forward	5' - TGAGACTCCGTTCTGGCCTC - 3′
	Reverse	3' - TAGCCCATGGTTCGTACTTCTC - 5′
mMCPT4	Forward	5' - TGACCGACACTGGCAAGATG - 3′
	Reverse	3' - GACTGGACGCACGTAGTCTC - 5′
TGF-β	Forward	5′- GAGTTTGTTATCTTTGCTGTCACA-3′
	Reverse	5′-TGAACCAAGGAGACGGAATACA-3′
VEGF	Forward	5' - CATCTTCAAGCCGTCCTGTGTG - 3′
	Reverse	3' - ACGACATTGCTACTTCGGGAC - 5′
PDGF	Forward	5' - GGTCAAACCTCTGAGGAAAGG - 3′
	Reverse	3' - AGTCTTTACTCGGGTCCCATGA - 5′
IGF-1	Forward	5' - ATGTACTGTGCCCCACTGAAG - 3′
	Reverse	3' - TGGACGTTTTGTAGCTTTGTG - 5′

TNF-α: Tumor necrosis factor-alpha, iNOS: Inducible nitric oxide synthase, Arg-1: Arginase- 1, MRC-1: Mannose receptor C-type-1, IL-33: Interleukin-33, mMCPT4: Mouse mast cell protease 4, TGF-β: Transforming growth factor-beta, VEGF: Vascular endothelial growth factor, PDGF: Platelet-derived growth factor, IGF-1: Insulin-like growth factor-1.

2.7. Flow cytometry analysis of leukocytes

After 15 days of the biocurative application, the samples were immediately cut using surgical scissors and submitted to an enzymatic digestion solution containing collagenase IV (40 U/mL) and DNAse (Sigma, 40 μg/mL) for 30 min at 37 °C, 200 rpm. Then, the samples were filtered through cell strainers of 70 μm and 40 μm. The resulting cell suspension was counted in an automatic cell counter (BioRad) and 0.5×10^6 cells from each mouse were incubated with anti-mouse antibodies (BD Biosciences) conjugated to fluorochromes for surface molecules. The conjugated antibodies used were CD45 (PE-Cy7), FcεRI (FITC), CD117 (APC), F4/80 (PerCP), CD11c (FITC) and CD206 (APC). The labeled cells were acquired on the FACSCanto II Cell Analyzer (BD Biosciences). The dispersions were analyzed using the FlowJo software (BD Biosciences).

2.8. Statistical analysis

For the analysis of the glycemic parameters of T1D, two-way ANOVA with Tukey post-test in time kinetics and unpaired t-test in the final glycemia were used. Two-way ANOVA was also used for comparative analysis of the areas of the lesions between the groups in the different periods evaluated. In the RT-PCR and flow cytometry analyzes, one-way ANOVA was used, followed by the Tukey post-test. The differences were considered statistically significant when the P-value was less than 5% ($P < 0.05$). These tests were performed using the statistical program Prism Graphpad 8.0.

3. Results

3.1. Clinical and metabolic parameters in STZ-induced T1D diabetic mice

Considering that the main characteristic of T1D is hyperglycemia, blood glucose was measured at 0, 5, 10, 15, and 30 days after STZ administration. Mice that exhibited blood glucose levels above 200 mg/dL in two consecutive measurements were considered diabetic. After 30 days of STZ administration, the incidence of T1D was 94.4% (n = 18, Fig. 1A). Glucose levels in mice submitted to T1D induction significantly increased from 10 days onwards, reaching a peak after 30 days (Fig. 1B). The hyperglycemic phenotype persisted throughout the 30-day period after T1D induction (Fig. 1C). Body weight remained steady in the first 15 days and then increased in the normoglycemic group (Fig. 1D). In the STZ-treated group, mice lost weight during the 5 initial days of induction, and body weight remained lower during the 30 day-period.

3.2. Decreased number of mast cells in the skin of diabetic mice

Wound induction consisted of removing 1 cm^2 of skin from the mice's dorsal surface. To determine differences in the number of skin MC between ND and T1D mice, the excised tissue was stained with toluidine blue. In addition, total RNA was extracted and mMCP4 gene expression, a connective tissue MC marker, was determined. A reduced number of MC was found in the skin of T1D mice compared to ND mice (Fig. 1E), which was corroborated by the lower mMCP4 gene expression (Fig. 1F).

3.3. Biodistribution kinetics and safety of MSCs after application of biocuratives into skin

Since we used a xenogeneic graft, i.e. a graft transplanted between members of two different species, before evaluating whether MSCs improve wound healing in diabetic mice, we analyzed the kinetics of biodistribution of these cells in the wound region and their potential to cause liver damage. No bioluminescent signals were detected in the groups that received intraperitoneal luciferin but did not receive MSCs. In the groups that received MSCs, the cells remained in the wound region, with a strong signal in the first day, which decreased thereafter (Fig. 2A and B). Moreover, there were no differences in serum AST (Fig. 2C) or ALT (Fig. 2D) between the ND groups that received biocuratives without or with MSCs, indicating that these cells do not induce hepatic damage.

3.4. Application of biocuratives with mesenchymal cells accelerated the healing process in diabetic mice

The graphs in Fig. 3 depict the wound closure process, represented by the wound area. Time 0, no wound closure, is represented as 100%, while total wound closure is represented by 0%. A slight improvement, with no statistically significant difference, was observed in healing in the ND + bMSC group when compared to the ND + b group (Fig. 3A and B). The detrimental effect of T1D on the healing processes is illustrated in Fig. 3C. ND mice healed faster than T1D mice, with significant differences from days 3–10. The application of biocuratives with MSCs improved healing in T1D mice, compared to T1D + b mice. Better healing in the T1D + bMSC group was observed from day 7, and accentuated 10 days after the beginning of the process, and are probably due to reparative effects of MSCs (Fig. 3D). In Fig. 3A, the representative images in the first row represent time 0 in mice from each experimental group. At this time, it is possible to visualize the biocuratives. The other images are representations of different times in the same mice. The representative images clearly indicate that the healing processes are similar, but the wound closure is different in specific time points. Notably, the wound of the T1D + b mouse had a delay to heal compared to the ND groups. However, there was greater wound closure in the T1D + bMSC mouse from days 7–10, when compared to that in T1D + b mice. On the fifteenth day, most mice, with exception of diabetic mice, had the wound completely closed (0% opening).

3.5. Biocuratives with mesenchymal cells reduced inflammation in diabetic mice

Fig. 4A (left panels) illustrates the morphology and the inflammatory infiltrate in the mice skin, after staining by H/E and at 15 days of healing. In the histological sections, the lesion area was identified by the reduced number of hair follicles and the presence of inflammatory cells (white double arrows) remaining in the granulation tissue. The ND + bMSC group showed better structural parameters, with a high number of hair follicles, greater organization of the fibrotic area, and reduced number of inflammatory cells, when compared with the ND + b group, suggesting that MSCs positively interfere with healing even in the absence of diabetes. In the T1D + bMSC group, the healing process was improved, evidenced by a higher number of hair follicles, apparent reduction in epidermal thickening, and reduced infiltration of inflammatory cells, when compared with T1D + b mice that presented impaired skin structure. Picrosirius staining (Fig. 4A, right panels) depicts the structure of collagen fibers, with type I collagen shown in red. In samples from ND groups and T1D + bMSC group, collagen fibers are

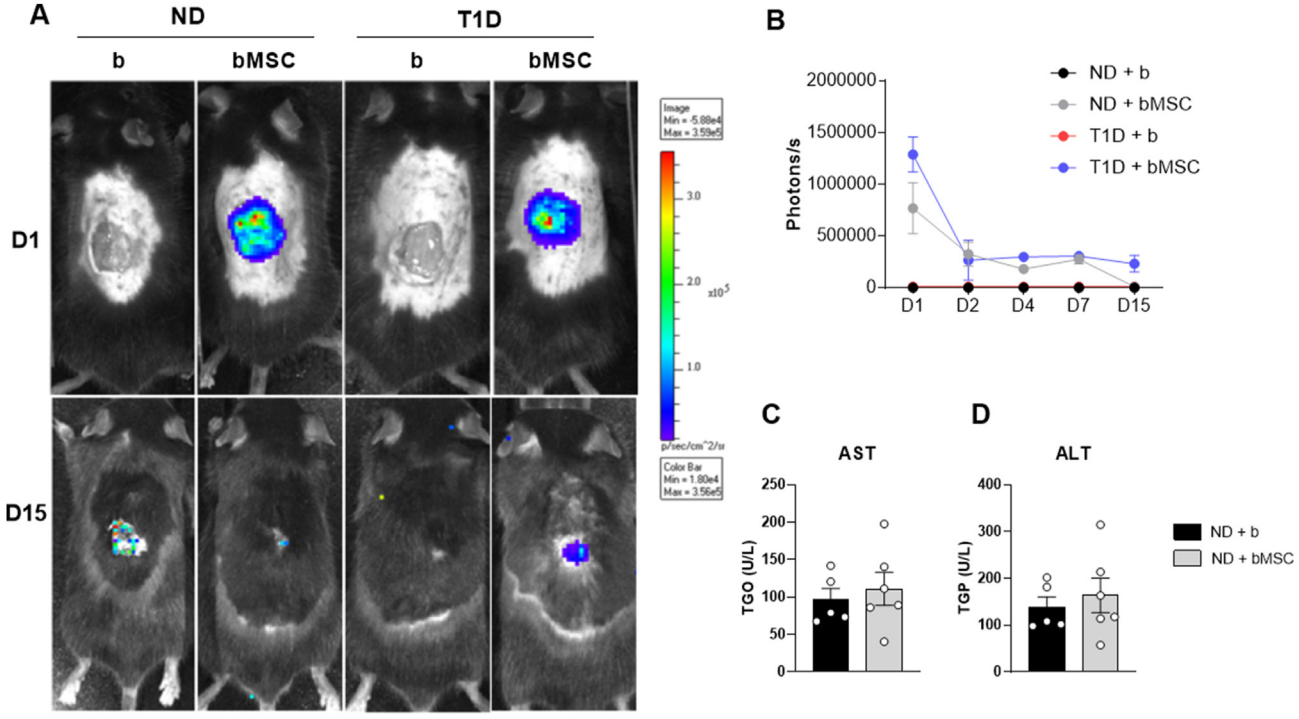

Fig. 2. MSCs in the biocuratives are restricted to the skin and do not induce liver toxicity. (A) Representative images of the bioluminescence of biocuratives with (bMSC) or without (b) MSCs transfected with the luciferase gene, obtained by an IVIS® equipment from PerkinElmer. **(B)** Bioluminescence readings, in photons/s, after application of biocuratives and luciferin at different time points. Quantification of AST **(C)** and ALT **(D)** enzymatic activity in the serum of mice that received biocuratives with or without MSC. Results are shown as mean ± SEM and are representative of one experiment (n = 5–6). Statistical significance was determined by unpaired t-test **(C, D)**. b: biocurative, bMSC: biocurative with MSC, ND: non-diabetic, T1D: type 1 diabetes, AST: aspartate transaminase, ALT: alanine transaminase.

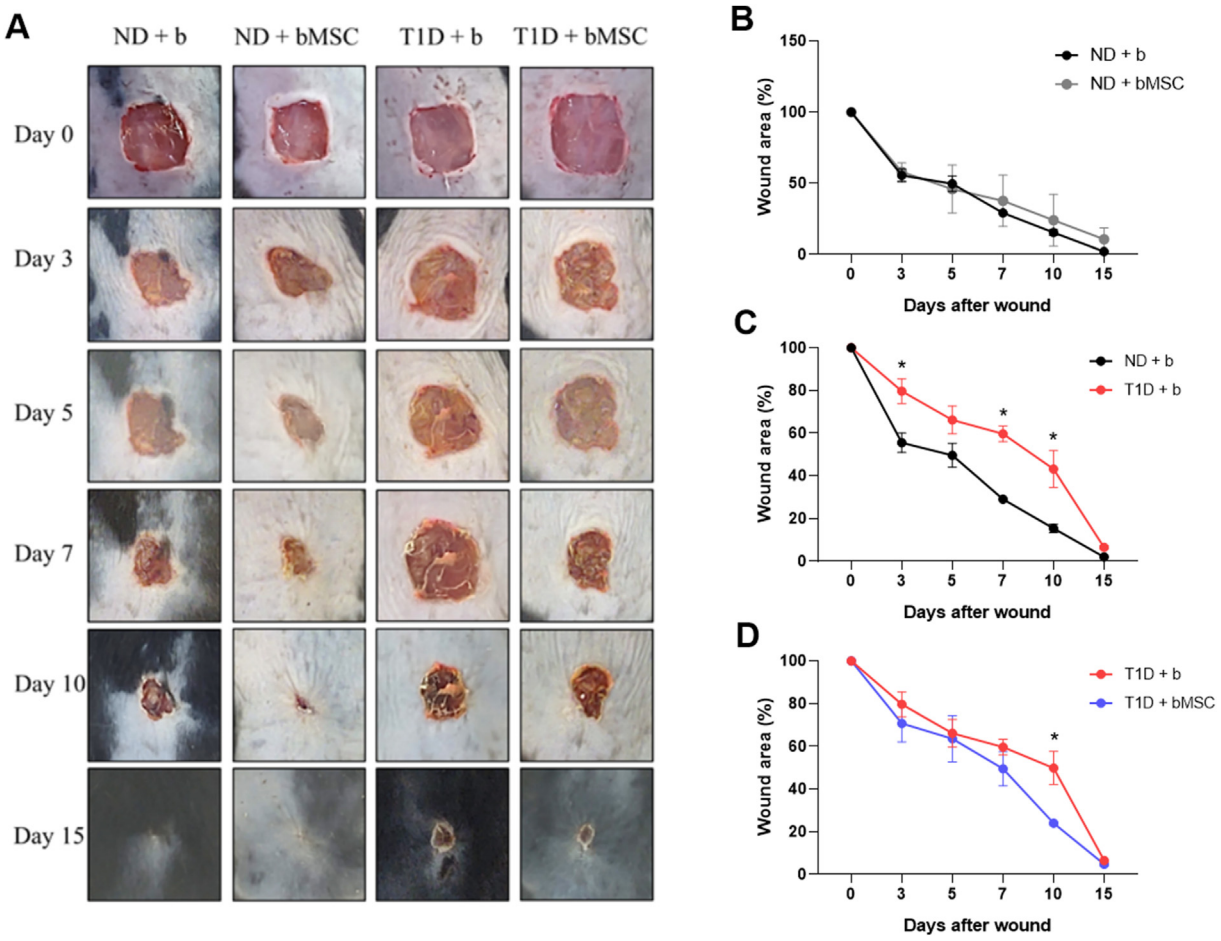

Fig. 3. Application of biocuratives with MSC accelerated the healing process in diabetic mice. (A) Representative photos depicting wound healing during 15 days. **(B, C and D)** Wound area percentages during the experimental period. Values are expressed as mean ± SEM and representative of two independent experiments (n = 3–6). Statistical significance was determined by two-way analysis of variance (ANOVA) and Sidak's multiple comparison test **(B, C, D).** *p < 0.05. b: biocurative, bMSC: biocurative with MSC, ND: non-diabetic, T1D: type 1 diabetes.

more organized, forming a reticular structure. On the other hand, in skin samples from T1D + b diabetic mice, the fibers are more scarce and thinner, forming parallel and inconsistent structures, which characterize the beginning of tissue remodeling. These results indicate that extracellular matrix deposition is impaired in the wound healing of diabetic mice, and suggest that biocuratives with mesenchymal cells reverses the diabetes-associated dysfunctional tissue repair.

3.6. Application of MSC biocuratives modulated gene expression of growth factors in the skin of diabetic mice

To evaluate whether MSCs modulate growth factors and thus contribute to improved wound healing, gene and protein expression of TGF-β, VEGF, PDGF and IGF1 was quantified. Gene expression of TGF-β, which is also an anti-inflammatory cytokine, was significantly higher in the T1D + bMSC group when compared to the T1D + b group (Fig. 4B). However, no differences were observed in the protein expression of this anti-inflammatory cytokine in the skin tissue (Fig. 4C). VEGF expression was significantly increase in T1D + b mice, when compared to ND + b, but significantly decreased in the T1D + bMSC group (Fig. 4D). No differences in VEGF protein expression were observed between the experimental groups (Fig. 4E). Furthermore, gene expression of PDGF (Fig. 4F) and IGF1 (Fig. 4G) was increased in the T1D + b group when compared to the ND + b group. Treatment of diabetic mice with the biocurative containing MSCs reduced the expression of PDGF and IGF1 when compared to the T1D + b group. These results indicate that T1D *per se* decreases TGF-β gene expression and increases VEGF, PDGF and IGF-1 gene expression in the skin, and that treatment with MSCs reverted gene expression of these growth factors.

3.7. Application of MSC biocuratives favored gene expression of M2 macrophages markers in diabetic mice

Gene expression of pro-inflammatory markers and repair/anti-inflammatory macrophages was determined at 15 days after wound. The expression of M1 macrophage markers, analyzed by TNF-α and iNOS gene expression [11], was significantly increased in the T1D + b group in comparison to the ND + b group (Fig. 5A and B). However, in the T1D + bMSC group, while the expression of TNF-α was potentiated (Fig. 5A), there was a significant reduction of iNOS gene expression in the skin, in comparison to the T1D + b group (Fig. 5B). To analyze the anti-inflammatory and regenerative M2 macrophage phenotype, the expression of the markers Arg-1, MRC-1, and IL-4 was determined [11]. Interestingly, increased ARG1 e MRC1 gene expression was observed in the T1D + bMSC group in comparison with the T1D + b group, suggesting that diabetic mice treated with MSCs have a higher proportion of M2 macrophages (Fig. 5C and D). In addition, the expression of IL-4,

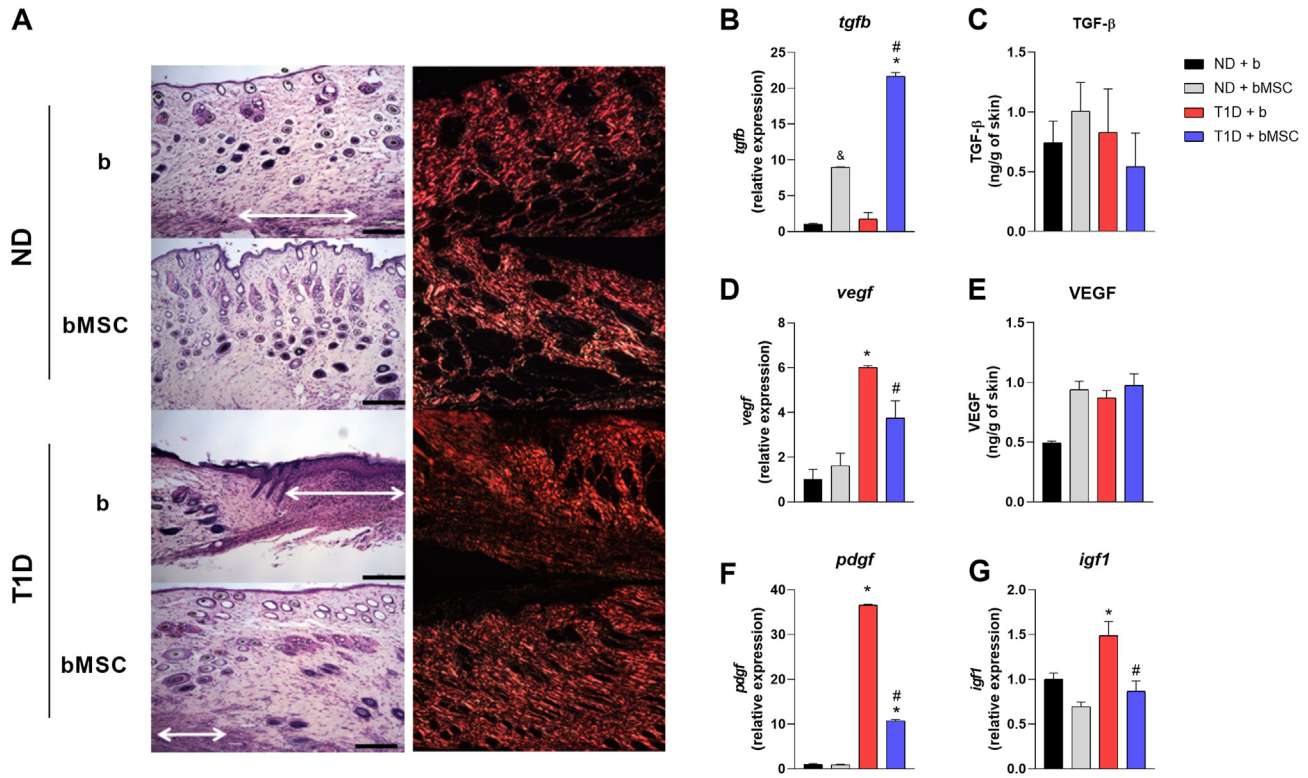

Fig. 4. MSCs increase collagen fibers and modulate growth factors gene expression in diabetic mice. (A) Representative photomicrographs of skin stained with HE (left panel) or picrosirius (right panel) for detection of collagen fibers. HE staining revealed infiltration of inflammatory cells (white double arrows) 15 days after the lesion induction. Magnification: × 100. Scale bar: 200 μm. The relative gene expression of Tgfb **(B)**, Vegf **(D)**, Pdgf **(F)** and Igf1 **(G)** in the skin after 15 days of wound was evaluated by RT-PCR. The production of TGF-β **(C)** and VEGF **(E)** in the skin homogenate was determined by ELISA. Results are shown as mean ± SEM and are representative of two independent experiments (n = 3–6). Statistical significance was determined by one-way analysis of variance (ANOVA) and Tukey's multiple comparison test **(B–G).** & ND + bMSC *vs* ND + b; *T1D *vs* ND; #T1D + bMSC vs T1D + b; $p < 0,05$. b: biocurative, bMSC: biocurative with MSC, ND: non-diabetic, T1D: type 1 diabetes.

which also contributes to healing through the induction of M2 macrophages, was extremely high in the diabetic mice, and the bMSC decreased IL-4 expression (Fig. 5E). The gene expression heat map of these markers is represented in Fig. 5F. Although treatment with MSCs modulated the expression of genes related to the M1 macrophage profile ($CD45^+$ $F4/80^+$ $CD11c^+$ cells) in diabetic mice, no statistical differences in the percentage or absolute number of M1 macrophages were observed (Fig. 5G). On the other hand, a trend to increased absolute number of M2 macrophages ($CD45^+$ $F4/80^+$ $CD206^+$ cells) was found in the T1D + bMSC group (Fig. 5H).

3.8. Application of biocuratives with mesenchymal cells restored the number of mast cells in the skin of diabetic mice

The role of MC in the healing process in diabetes is not yet fully elucidated [41]. Mast cells express ST2, a component of the IL-33 receptor, which is also involved in wound healing [17]. Therefore, gene expression of IL-33 and the MC marker mMCP4 in the skin of mice after wound and application of MSCs were evaluated. Gene expression of IL-33 (Fig. 6A) and mMCP4 (Fig. 6B) was increased in the diabetic mice treated with MSC when compared with the T1D + b group. In addition, when evaluating the MC population ($CD45^+$ $Fc\varepsilon RI^+$ $CD117^+$) by flow cytometry, while the treatment with MSC did not change the percentage, the absolute number of MC increased slightly in the skin of diabetic mice at the evaluated time point (Fig. 6C). To confirm these data, toluidine blue staining was used and the skin MC number was quantified. MSC treatment restored the number of MC in the skin of diabetic mice when compared with T1D + b mice (Fig. 6D and E), suggesting that the MC – IL-33 axis, with potential effects of IL-33 on the migration/activation of skin MC, may be important in driving MSCs-induced wound healing in diabetic mice.

4. Discussion

In the present study, mice submitted to STZ-induced T1D exhibited a 94.4% incidence of disease, high blood glucose levels from days 10–30 after the start of the STZ protocol, weight loss, defective wound healing, and skin phenotype alterations [12].

Wound healing in people affected by T1D is often extremely dysregulated, suggesting important abnormalities in the skin of these individuals, compared to healthy individuals. Diabetic mice exhibit alterations in pro and anti-inflammatory cytokines expression and proliferative capacity in skin cells, such as keratinocytes [42] and fibroblasts [14]. An increased number and degranulation of mast cells was reported in skin biopsies of the forearms of diabetic patients compared to normal individuals. However, only mast cell degranulation was increased in the skin biopsies of the feet, the main site of occurrence of wounds in diabetic patients [43]. There is no consensus in the literature on potential changes in the number and function of skin-resident mast cells in T1D individuals and experimental animals.

The present study shows that an intrinsic defect in the number of mast cells in the skin may be a contributing factor in the deficient healing in T1D mice. The number of mast cells is reduced in the skin of T1D mice, when compared to ND mice, and this phenotype is corroborated by the lower skin gene expression of the connective tissue mast cell marker mcpt4. Under homeostasis, the cytokine IL-33 is constitutively expressed in the cytosol, stored in the nucleus, and secreted by different skin cells, such as keratinocytes and mast

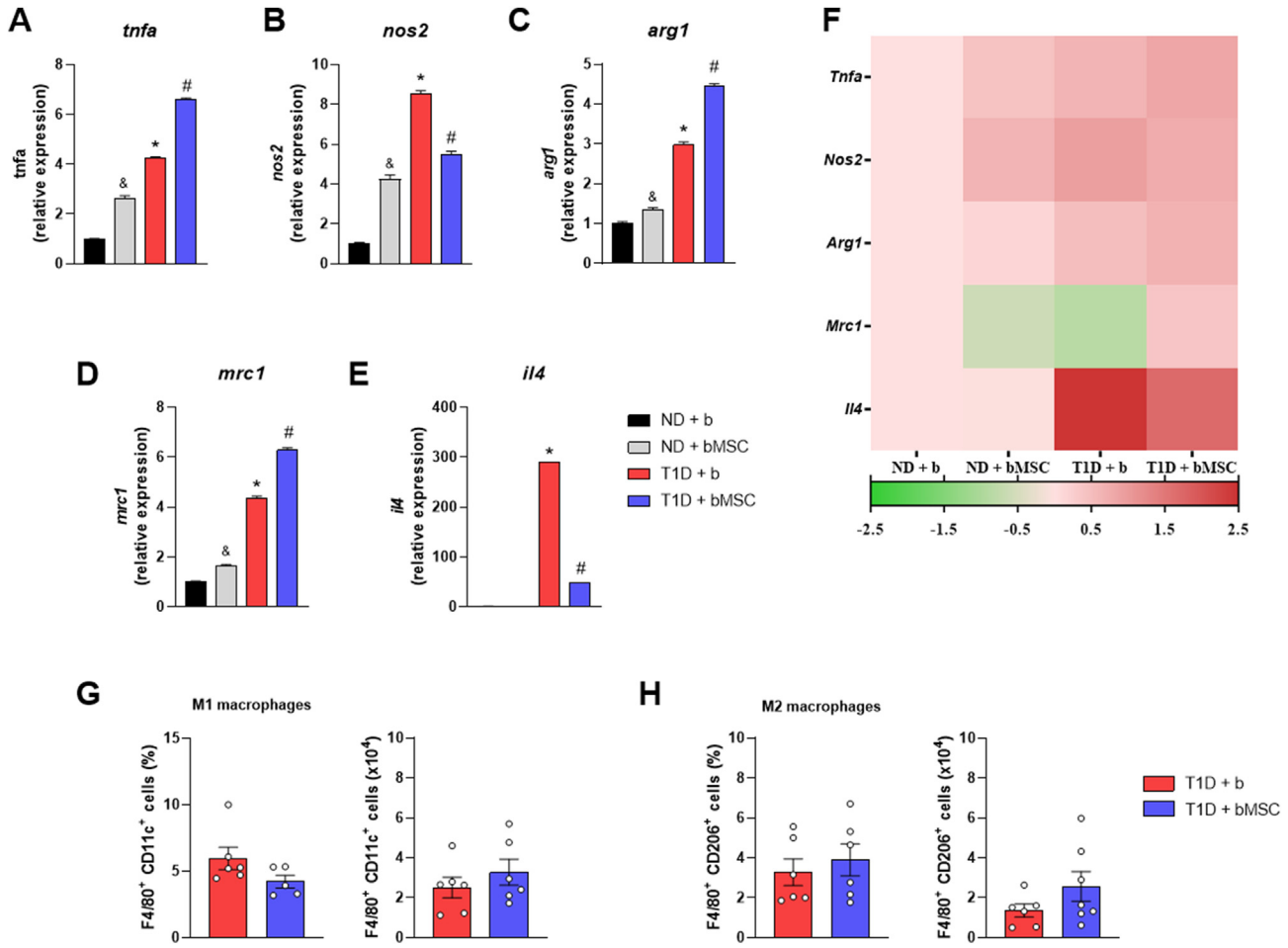

Fig. 5. MSC increase the gene expression of markers associated with M2 macrophages in skin of diabetic mice. The relative expression of Tnfα **(A)**, Nos2 **(B)**, Arg1 **(C)**, Mrc1 **(D)** and Il4 **(E)** genes in the skin after 15 days of wound was evaluated by RT-PCR. **(F)** Heatmap with compilation of the relative expressions of the evaluated genes. Percentage and absolute number of M1 **(G)** and M2 macrophages **(H)** were quantified by flow cytometry in scar tissue harvested 15 days after wound induction. Results are shown as mean ± SEM and are representative of two independent experiments (n = 3–6). Statistical significance was determined by one-way analysis of variance (ANOVA) and Tukey's multiple comparison test **(B–G).** $^{\&}$ ND + bMSC *vs* ND + b; *T1D *vs* ND; $^{\#}$T1D + bMSC *vs* T1D + b; $p < 0,05$. b: biocurative, bMSC: biocurative with MSC, ND: non-diabetic, T1D: type 1 diabetes.

cells [16,44], and its administration proved to have an important role in skin healing in a murine model [45]. Additionally, IL-33 is released as an alarmin by infected or necrotic cells. As IL-33 participates in the survival, maturation, and activation of skin mast cells [18], a defect in the number of mast cells in the skin of diabetic mice may be attributed to lower IL-33 availability, which in turn would be detrimental to healing. Activated MC secrete several cytokines, including TGF-β, whose gene expression is increased in the group with T1D that received biodressing containing MSC in our model. TGF-β coordinates the induction of MC-induced regulatory T cells (T reg) in an allergy model [46], for example, and these cells are important in the repair of skin wound injuries [47]. However, it was not possible to associate the decreased number of mast cells to the reduced IL-33 gene expression in the skin of STZ mice [48].

Our findings confirm that STZ-induced T1D impairs wound closure and that the application of MSC biocuratives restores the ability of these wounds to heal at an earlier time. The improved skin wound healing after MSC administration has been reported in other models and different mechanisms were suggested [49–51]. When comparing the ND and T1D groups that were not treated with MSCs, the deleterious effect of T1D on regenerative healing processes was very clear. Of importance, the MSC biocuratives significantly improved the kinetics of wound closure in the diabetic animals.

MSCs administered by biocuratives stimulate the immune system, which in turn has a direct impact on healing [31]. Inflammation is necessary in the first phase of the healing process. However, when it persists for a long time, it compromises the progression of other regenerative phases. One of the reasons for healing failure in diabetic individuals is the persistent inflammation, which occurs due to the reduced ability of these individuals to produce anti-inflammatory cytokines, growth factors, and differentiation of M2 macrophages, all responsible for controlling inflammation [8,10,21]. The cellular profile of macrophages is very important in the balance of inflammation, and after the inflammatory phase of healing, macrophages differentiate to the M2 phenotype that secrete growth factors and anti-inflammatory cytokines [52]. Diabetic individuals undergo several metabolic and inflammatory changes, which impair macrophage differentiation, favoring the M1 phenotype in detriment of M2 macrophages, delaying the resolution of inflammation and wound healing [11,53].

Here, we confirmed that diabetes and wound healing is also related to imbalanced M1 and M2 macrophages markers. T1D mice exhibited increased expression of the classic markers of M1 and M2 macrophage phenotypes, compared to other experimental groups. This is probably due to the unsuccessful attempt of the diabetic wound microenvironment to restore homeostasis of healing processes, increasing signals related to both cellular phenotypes.

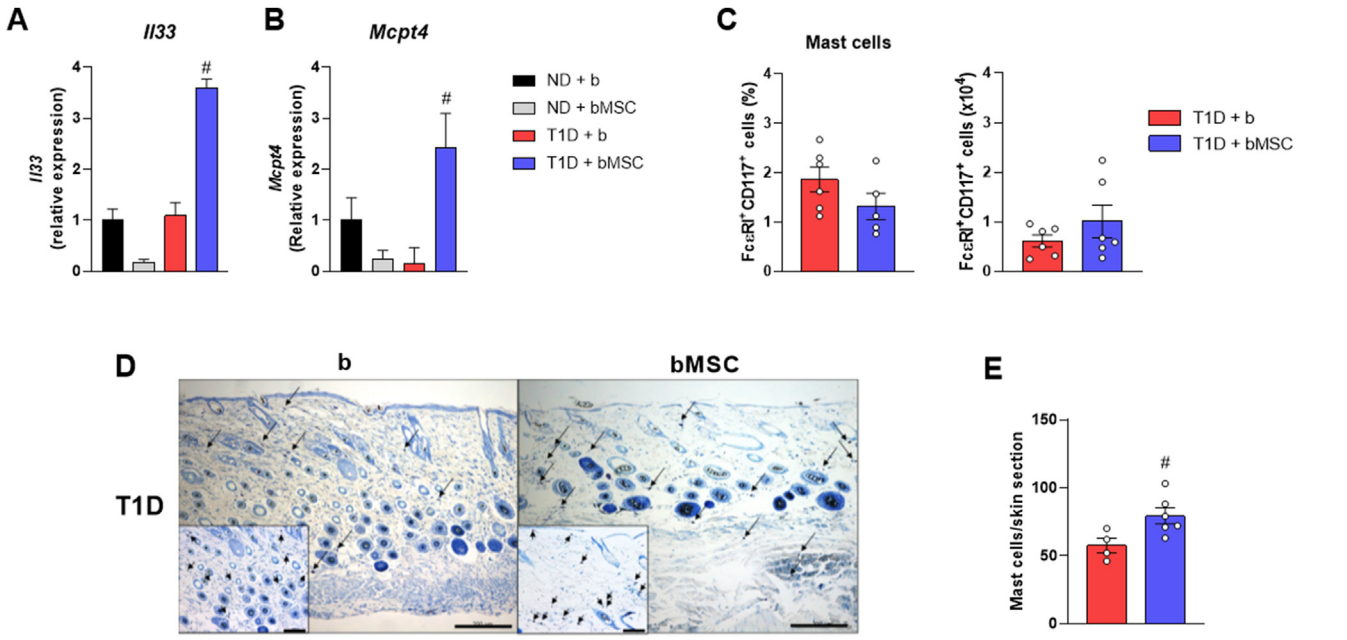

Fig. 6. MSC application restores the amount of MC in the skin of diabetic mice. (A) Il33 and **(B)** Mmcpt4 gene relative expression evaluated in the skin after 15 days of wound by RT-PCR. **(C)** Percentage and absolute number of mast cells quantified by flow cytometry in scar tissue harvested 15 days after wound induction. **(D)** Representative photomicrographs of skin sections stained with toluidine blue for mast cell labeling. Magnification 200x (scale bar: 100 μm). **(E)** Mast cell counts per skin section stained with toluidine blue. Results are shown as mean ± SEM and are representative of two independent experiments (n = 3–6). Statistical significance was determined by one-way analysis of variance (ANOVA) and Tukey's multiple comparison test **(A, B)** or unpaired t-test **(C, E)**. $^{\&}$ ND + bMSC *vs* ND + b; *T1D *vs* ND; $^{\#}$T1D + bMSC *vs* T1D + b; $p < 0,05$. b: biocurative, bMSC: biocurative with MSC, ND: non-diabetic, T1D: type 1 diabetes.

Comparing the ND + b and ND + bMSC groups, MSCs, by direct or indirect manners, altered the presence of all analyzed macrophage markers, except IL-4, independently of previous disease or inflammatory condition. The MSCs increased the expression of M1 markers, probably due to their xenogeneic effect. Surprisingly, bMSC administration increased TNF-α gene expression and reduced iNOS expression, M1 macrophage markers, in diabetic mice. On the other hand, M2 markers, Arg 1 and Mrc1, were upregulated in diabetic mice that received bMSC. In agreement, pretreatment of mesenchymal cells with IFN-y and TNF-α improves their healing potential by promoting polarization of M2 macrophages [54]. Similarly, it has already been shown that peritoneal macrophages stimulated with LPS and then stimulated with MSCs acquire the M2 profile, in a TGF-β dependent manner. Furthermore, the treatment of these cells with TGF-β activated the phospho-Akt/FoxO 1 pathway, an important signal for the regulatory effects of TGF-b [55]. Thus, MSCs upregulate the expression of repair and anti-inflammatory M2 macrophage markers, in agreement with the prevalence of this cell subtype in the repaired tissue. The role of MCs in skin healing in controversial [56]. We observed less mast cells in the skin of diabetic mice versus non-diabetic mice, and bMSC administration increased the number of mast cells, confirmed by toluidine blue staining, and il-33 and Mmcpt4 gene expression. These data suggest that the mast cell-IL-33 axis is important to improve healing in T1D. In agreement with our results, diabetic mice deficient in mast cells show impaired wound healing, and topical administration of substance P, which together with IL-33 induces VEGF, accelerates healing in a mast cell-dependent manner [57]. In addition, the topical use of mast cell stabilizers also accelerates the healing of skin wounds in diabetic mice [58]. The healing activity of mast cells is also observed in models of skin infection, in which mast cells are essential for controlling bacterial load and wound closure [59]. Also, we detected that Il-33 gene expression is significantly higher in the T1D + bMSC group, indicating a possible synergy between this cytokine and mast cells.

The actions of MSCs on the immune system, in addition to actions on other cells in the wound environment, improved collagen deposition in diabetic mice [60]. We showed that T1D + b group has a great loss in the amount of type I collagen deposition and its fibers are not reticular, in comparison to the non-diabetic group. Collagen deposition defects in diabetic mice can be explained by the increased tissue damage caused by inflammation, reducing the ability of granulation tissue cells to produce collagen molecules and increasing the expression of metalloproteinases that degrade these proteins [8,61]. Here, we observed that T1D + bMSC exhibited improved collagen deposition, suggesting that this treatment reduces tissue damage by modulating the secretion of matrix proteins and contributing to skin healing.

The secretion of collagen and other components of the cellular matrix is mediated by growth factors, such as TGF-β, important in the maintenance of proliferation and differentiation of cells in the wound environment [62,63]. We observed a positive regulation of TGF-β gene expression in the T1D + bMSCs. TGF-β stimulates the proliferation of fibroblasts and has an anti-inflammatory action [64], being one of the potential factors responsible for the improvement of the healing in the T1D + bMSC group. The expression of VEGF, PDGF, and IGF-1 is essential for the proliferation of endothelial cells, fibroblasts, and keratinocytes [21]. In our model, bMSC administration reduced the expression of these factors when compared to the T1D + b group. Contrary to our data, systemic or topical administration of MSC increased the production of VEGF, and concomitantly improved wound healing in mice with type 2 diabetes [65].

Taken together, our findings suggest that xenogeneic MSCs applied by hydrogel biocuratives reverse the impaired healing in diabetic animals, by stimulating mast cells and M2 macrophages in the skin and by increasing the secretion of pro-fibrotic and pro-

healing factors. Therefore, our data support that MSCs biocuratives provide a promising strategy in the treatment of skin wounds associated with T1D.

Ethics approval

The project and experimental animal procedures were approved by the Ethics Committee in the Animal Use (CEUA) of the Ribeirão Preto Medical School, University of São Paulo (Process number: 209/2019).

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Authors' contributions

GMCM designed and performed the experiments, analyzed the results, and wrote the manuscript. JE-O performed the experiments and analyzed the results. JBG, ISP, and VFR performed the experiments. BB discuss experimental protocols and helped in the images analysis. DMCF and LEBS transfected the cells with luciferase and assisted in performing the bioluminescence experiment. HGR discussed experimental protocols, helped in image analysis and critically analyzed the manuscript. VDB and JSS provided intellectual assistance. SGM contributed to the execution and analysis of skin histology. RCT corrected and critically evaluated this manuscript. AOM and CC-O supplied the biocuratives containing mesenchymal stem cells and assisted in the wound induction model. DC designed and supervised the research, analyzed data, and wrote the paper.

Declaration of competing interest

CCO and AOM are partners is *In Situ Cell Therapy* and are named as inventors of a provisional patent directed at this manuscript, which is solely owned by *In Situ Cell Therapy*.

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References

[1] Fowler MJ. Microvascular and macrovascular complications of diabetes [Internet]. Clin Diabetes 2008;26(2):77–82. [cited 2019 Jul 22]. Available from: http://clinical.diabetesjournals.org/cgi/doi/10.2337/diaclin.26.2.77.
[2] Rask-Madsen C, King GL. Vascular complications of diabetes: mechanisms of injury and protective factors. Cell Metabol 2013:20–33.
[3] Brem H, Tomic-Canic M. Cellular and molecular basis of wound healing in diabetes. J Clin Invest 2007:1219–22.
[4] Bender KP, Tonon da Luz S, Monchizuki L, D'Orsi E. Burden of disease from lower limb amputations attributable to diabetes mellitus in Santa Catarina State, Brazil, 2008-2013. Carga reports public heal [Internet] 2008;34:13. Available from: https://doi.org/10.1590/0102-311X00013116.
[5] Gamba MA, Gotlieb SLD, Pimentel Bergamaschi D, Vianna LAC. Amputações de extremidades inferiores por diabetes mellitus: estudo caso-controle. Rev Saude Publica 2004;38:399–404.
[6] Pedrosa H, Vilar L, Boulton AJM. Neuropatias e pé diabético. AC Farm 2014;1:328.
[7] De Mendonça RJ, Coutinho-Netto J. Aspectos celulares da cicatrização. An Bras Dermatol 2009:257–62.
[8] Turabelidze A, Dipietro LA. Inflammation and wound healing. Endod Top 2012;24:26–38.
[9] Eming SA, Martin P, Tomic-Canic M. Wound repair and regeneration: mechanisms, signaling, and translation. Sci Transl Med 2014;6(265):265sr6.
[10] Serra MB, Barroso WA, da Silva NN, Silva SN, Borges ACR. Abreu IC, Borges MOR. From Inflammation to Current and Alternative Therapies Involved in Wound Healing. Int J Inflam 2017;2017:3406215. https://doi.org/10.1155/2017/3406215.
[11] Boniakowski AE, Kimball AS, Jacobs BN, Kunkel SL, Gallagher KA. Macrophage-mediated inflammation in normal and diabetic wound healing. J Immunol [Internet] 2017;199:17–24. Available from: https://www.jimmunol.org/content/199/1/17.
[12] Andrade TAM, Masson-Meyers DS, Caetano GF, Terra VA, Ovidio PP, Jordão-Júnior AA, et al. Skin changes in streptozotocin-induced diabetic rats. Biochem Biophys Res Commun 2017;490:1154–61. Elsevier B.V.
[13] Kolluru GK, Bir SC, Kevil CG. Endothelial dysfunction and diabetes: effects on angiogenesis, vascular remodeling, and wound healing. Int J Vasc Med 2012;2012:918267. https://doi.org/10.1155/2012/918267.
[14] Lerman OZ, Galiano RD, Armour M, Levine JP, Gurtner GC. Cellular dysfunction in the diabetic fibroblast: impairment in migration, vascular endothelial growth factor production, and response to hypoxia. Am J Pathol 2003;162:303–12.
[15] Lobmann R, Ambrosch A, Schultz G, Waldmann K, Schiweck S, Lehnert H. Expression of matrix-metalloproteinases and their inhibitors in the wounds of diabetic and non-diabetic patients. Diabetologia 2002;45:1011–6.
[16] Oshio T, Komine M, Tsuda H, Tominaga S ichi, Saito H, Nakae S, et al. Nuclear expression of IL-33 in epidermal keratinocytes promotes wound healing in mice. J Dermatol Sci [Internet] 2017;85:106–14. Elsevier Ireland Ltd cited 2020 Oct 1Available from: https://www.jdsjournal.com/article/S0923181116308805/fulltext.
[17] He R, Yin H, Yuan B, Liu T, Luo L, Huang P, et al. IL-33 improves wound healing through enhanced M2 macrophage polarization in diabetic mice. Mol Immunol 2017;90:42–9. Elsevier.
[18] Méndez-Enríquez E, Hallgren J. Mast cells and their progenitors in allergic Asthma. Front. Immunol. Frontiers Media S.A.; 2019.
[19] Theoharides TC, Zhang B, Kempuraj D, Tagen M, Vasiadi M, Angelidou A, et al. IL-33 augments substance P-induced VEGF secretion from human mast cells and is increased in psoriatic skin. Proc natl acad sci U S A [internet] 2010;107:4448–53. National Academy of Sciences [cited 2021 Feb 13] Available from:/pmc/articles/PMC2840132/.
[20] Rodrigues M, Kosaric N, Bonham CA, Gurtner GC. Wound healing: a cellular perspective. Physiol Rev 2019;99:665–706.
[21] Larouche J, Sheoran S, Maruyama K, Martino MM. Immune regulation of skin wound healing: mechanisms and novel therapeutic targets. Adv Wound Care 2018;7:209–31.
[22] Kanji S, Das H. Advances of stem cell therapeutics in cutaneous wound healing and regeneration. Mediators Inflamm 2017;5217967:1–14. https://doi.org/10.1155/2017/5217967.
[23] Yolanda M-M, Maria A-V, Amaia F-G, Marcos P-B, Lopez Silvia P, Dolores E, et al. Adult stem cell therapy in chronic wound healing menendez-menendez. J Stem Cell Res Ther 2014;4:162.
[24] Kinoshita K, Kuno S, Ishimine H, Aoi N, Mineda K, Kato H, et al. Therapeutic potential of adipose-derived SSEA-3-positive muse cells for treating diabetic skin ulcers. Stem Cells Transl Med 2015;4:146–55.
[25] Noronha Nc NDC, Mizukami A, Caliári-Oliveira C, Cominal JG, Rocha JLM, Covas DT, et al. Priming approaches to improve the efficacy of mesenchymal stromal cell-based therapies [Internet]. Stem Cell Res Ther 2019. Available from: https://doi.org/10.1186/s13287-019-1224-y.
[26] Badillo AT, Redden RA, Zhang L, Doolin EJ, Liechty KW. Treatment of diabetic wounds with fetal murine mesenchymal stromal cells enhances wound closure. Cell Tissue Res 2007;329:301–11.
[27] Di Rocco G, Gentile A, Antonini A, Ceradini F, Wu JC, Capogrossi MC, et al. Enhanced healing of diabetic wounds by topical administration of adipose tissue-derived stromal cells overexpressing stromal-derived factor-1: biodistribution and engraftment analysis by bioluminescent imaging. Stem Cell Int 2011;2011.
[28] McFarlin K, Gao X, Liu YB, Dulchavsky DS, Kwon D, Arbab AS, et al. Bone marrow-derived mesenchymal stromal cells accelerate wound healing in the rat. Wound repair Regen Off Publ Wound Heal Soc [and] Eur Tissue Repair Soc. United States 2006;14:471–8.
[29] Nourian Dehkordi A, Mirahmadi Babaheydari F, Chehelgerdi M, et al. Skin tissue engineering: wound healing based on stem-cell-based therapeutic strategies. Stem Cell Res Ther 2019;10:1–20. https://doi.org/10.1186/s13287-019-1212-2.
[30] Wu Y, Chen L, Scott PG, Tredget EE. Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. Stem Cell 2007;25:2648–59.
[31] Teng M, Zhang H. The role of mesenchymal stromal cells in skin wound healing. Biol Ther Appl Mesenchymal Cells 2016:845–56.
[32] Otero-Viñas M, Falanga V. Mesenchymal stem cells in chronic wounds: the spectrum from basic to advanced therapy. Adv Wound Care 2016:149–63.
[33] Wang Y, Chen X, Cao W, Shi Y. Plasticity of mesenchymal stem cells in immunomodulation: pathological and therapeutic implications. Nat Immunol 2014:1009–16. Nature Publishing Group.
[34] Nuschke A. Activity of mesenchymal stem cells in therapies for chronic skin wound healing. Organogenesis 2014:29–37. Landes Bioscience.
[35] Chen S, Shi J, Zhang M, Chen Y, Wang X, Zhang L, et al. Mesenchymal stem cell-laden anti-inflammatory hydrogel enhances diabetic wound healing. Sci Rep 2015;5.
[36] Deng C, Wang L, Feng J, Lu F. Treatment of human chronic wounds with autologous

extracellular matrix/stromal vascular fraction gel: a STROBE-compliant study. Med (United States) 2018;97.
[37] Lee DE, Ayoub N, Agrawal DK. Mesenchymal stem cells and cutaneous wound healing: novel methods to increase cell delivery and therapeutic efficacy. Stem Cell Res Ther 7, 37 (2016), 1-8. https://doi.org/10.1186/s13287-016-0303-6
[38] Furman BL. Streptozotocin-induced diabetic models in mice and rats. Curr Protoc Pharmacol 2015;70:5.47.1–5.47.20.
[39] Burger B, Kühl CMC, Candreva T, Cardoso R da S, Silva JR, Castelucci BG, et al. Oral administration of EPA-rich oil impairs collagen reorganization due to elevated production of IL-10 during skin wound healing in mice. Sci Rep [Internet] 2019;9:1–13 [cited 2021 Feb 13].Available from: https://doi.org/10.1038/s41598-019-45508-1. Nature Publishing Group cited 2021 Feb 8] Available from:.
[40] Francesko A, Petkova P, Tzanov T. Hydrogel dressings for advanced wound management. Curr Med Chem. United Arab Emirates 2018;25:5782–97.
[41] Komi DEA, Khomtchouk K, Santa Maria PL. A review of the contribution of mast cells in wound healing: involved molecular and cellular mechanisms [internet]. Clin. Rev. Allergy Immunol. Springer 2020:298–312 [cited 2021 Feb 13]. Available from: https://link.springer.com/article/10.1007/s12016-019-08729-w.
[42] Okano J, Kojima H, Katagi M, Nakagawa T, Nakae Y, Terashima T, et al. Hyperglycemia induces skin barrier dysfunctions with impairment of epidermal integrity in non-wounded skin of type 1 diabetic mice. PLoS One 2016;11:1–22.
[43] Tellechea A, Leal EC, Kafanas A, Auster ME, Kuchibhotla S, Ostrovsky Y, et al. Mast cells regulate wound healing in diabetes. Diabetes 2016 Jul;65(7):2006–19. https://doi.org/10.2337/db15-0340.
[44] Carlos D, Spiller F, Souto FO, Trevelin SC, Borges VF, de Freitas A, et al. Histamine H 2 receptor signaling in the pathogenesis of sepsis: studies in a murine diabetes model. J Immunol 2013;191:1373–82.
[45] Yin H, Li X, Hu S, Liu T, Yuan B, Gu H, et al. IL-33 accelerates cutaneous wound healing involved in upregulation of alternatively activated macrophages. Mol Immunol. England 2013;56:347–53.
[46] Morita H, Arae K, Unno H, Miyauchi K, Toyama S, Nambu A, et al. An interleukin-33-mast cell-interleukin-2 Axis suppresses papain-induced allergic inflammation by promoting regulatory T cell numbers. Immunity. United States 2015;43:175–86.
[47] Nosbaum A, Prevel N, Truong H-A, Mehta P, Ettinger M, Scharschmidt TC, et al. Cutting edge: regulatory T cells facilitate cutaneous wound healing. J Immunol. United States 2016;196:2010–4.
[48] Travers J, Rochman M, Miracle CE, Habel JE, Brusilovsky M, Caldwell JM, et al. Chromatin regulates IL-33 release and extracellular cytokine activity. Nat Commun [Internet] 2018;9:1–15. Springer US Available from: https://doi.org/10.1038/s41467-018-05485-x.
[49] Munir S, Basu A, Maity P, Krug L, Haas P, Jiang D, et al. TLR4-dependent shaping of the wound site by MSCs accelerates wound healing. EMBO Rep 2020;21:e48777.
[50] Putra A, Alif I, Hamra N, Santosa O, Kustiyah AR, Muhar AM, et al. MSC-released TGF-β regulate α-SMA expression of myofibroblast during wound healing. J Stem Cells Regen Med 2020;16:73–9.
[51] Jiang T, Wang Z, Sun J. Human bone marrow mesenchymal stem cell-derived exosomes stimulate cutaneous wound healing mediates through TGF-β/Smad signaling pathway. Stem Cell Res Ther [Internet 2020;11:198. Available from: https://doi.org/10.1186/s13287-020-01723-6.
[52] Krzyszczyk P, Schloss R, Palmer A, Berthiaume F. The role of macrophages in acute and chronic wound healing and interventions to promote pro-wound healing phenotypes. Front Physiol. 2018 May 1;9:419, 1-22. https://doi.org/10.3389/fphys.2018.00419.
[53] Delavary BM, van der Veer WM, van Egmond M, Niessen FB, Beelen RHJ. Macrophages in skin injury and repair. Immunobiology 2011:753–62.
[54] Liu C, Lu Y, Du P, Yang F, Guo P, Tang X, et al. Mesenchymal stem cells pretreated with proinflammatory cytokines accelerate skin wound healing by promoting macrophages migration and M2 polarization. Regen Ther [Internet] 2022;21:192–200. Available from: https://www.sciencedirect.com/science/article/pii/S2352320422000621.
[55] Liu F, Qiu H, Xue M, Zhang S, Zhang X, Xu J, et al. MSC-secreted TGF-β regulates lipopolysaccharide-stimulated macrophage M2-like polarization via the Akt/FoxO 1 pathway. Stem Cell Res Ther [Internet] 2019;10:345. Available from: https://doi.org/10.1186/s13287-019-1447-y.
[56] Ud-Din S, Wilgus TA, Bayat A. Mast cells in skin scarring: a review of animal and human research. Front Immunol [Internet] 2020:11. Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2020.552205.
[57] Tellechea A, Leal EC, Kafanas A, Auster ME, Kuchibhotla S, Ostrovsky Y, et al. Mast cells regulate wound healing in diabetes. Diabetes [Internet]. American Diabetes Association Inc. 2016:2006–19 [cited 2021 Feb 7]. p. Available from:/=pmc/articles/PMC4915574/?report=abstract.
[58] Tellechea A, Bai S, Dangwal S, Theocharidis G, Nagai M, Koerner S, et al. Topical application of a mast cell stabilizer improves impaired diabetic wound healing. J Invest Dermatol. United States 2020;140:901–911.e11.
[59] Zimmermann C, Troeltzsch D, Giménez-Rivera VA, Galli SJ, Metz M, Maurer M, et al. Mast cells are critical for controlling the bacterial burden and the healing of infected wounds. Proc Natl Acad Sci U S A 2019;116:20500–4.
[60] Zgheib C, Hodges M, Hu J, Beason DP, Soslowsky LJ, Liechty KW, et al. Mechanisms of mesenchymal stem cell correction of the impaired biomechanical properties of diabetic skin: the role of miR-29a. Wound repair Regen Off Publ Wound Heal Soc [and] Eur Tissue Repair Soc 2016;24:237–46.
[61] Xue M, Jackson CJ. Extracellular matrix reorganization during wound healing and its impact on abnormal scarring. Adv Wound Care. Mary Ann Liebert Inc 2015;4:119–36.
[62] Varga J, Rosenbloom J, Jimenez SA. Transforming growth factor beta (TGF beta) causes a persistent increase in steady-state amounts of type I and type III collagen and fibronectin mRNAs in normal human dermal fibroblasts. Biochem J 1987;247:597–604.
[63] Liarte S, Bernabé-García Á, Nicolás FJ. Role of TGF-β in skin chronic wounds: a keratinocyte perspective. Cells 2020;9.
[64] Lichtman MK, Otero-Vinas M, Falanga V. Transforming growth factor beta (TGF-β) isoforms in wound healing and fibrosis. Wound Repair Regen 2016 Mar;24(2):215–22. https://doi.org/10.1111/wrr.12398.
[65] Yan J, Liang J, Cao Y, El Akkawi MM, Liao X, Chen X, et al. Efficacy of topical and systemic transplantation of mesenchymal stem cells in a rat model of diabetic ischemic wounds. Stem Cell Res Ther 2021;12:220.