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Mesenchymal stromal cells suspended in a platelet lysate gel improve wound healing in mice

Luciana da Rosa Zinn Sostizzo[1,2], Raul Marques Rodrigues[2], Tuane Nerissa Alves Garcez[1], Victor de Mello Palma[3], Annelise Martins Pezzi da Silva[1], Vanessa de Souza Valim[1], Ana Helena da Rosa Paz[1,4], Anelise Bergmann Araújo[1], Alessandra Aparecida Paz[1], Lucia Silla[1,2]*

ABSTRACT

Objective: To assess the safety and efficacy of topical wound treatment with mesenchymal stromal cells derived from bone marrow cultured and suspended in platelet lysate. **Method:** A prospective, interventional, controlled study was conducted with 42 C57BL/6 mice with induced excisional wounds. They were divided into four treatment groups: hydrogel (standard wound treatment), saline gel (control), platelet lysate gel, and platelet lysate gel containing mesenchymal stromal cells. Immunohistochemistry analyses were performed on day 7 (n=5) and day 13 (n=7), and safety on day 21 (n=30). Follow-up was conducted through clinical (adverse events, weight, pain, behavior, wound size reduction) and histological (healing, vascularization, collagenization) assessments. **Results:** Regarding safety, no adverse events or physiological changes related to the treatment were identified. The group treated with cells showed better results in wound healing through macro and microscopic assessments, followed by the platelet lysate gel group. **Conclusion:** The topical application of mesenchymal stromal cells was safe and showed favorable results.

KEYWORDS: Wound Healing. Mesenchymal Stromal Cell. Cell Therapy. Platelet Lysate. Stomatherapy.

Células estromais mesenquimais suspensas em gel de lisado de plaquetas melhoram a cicatrização de feridas em camundongos

RESUMO

Objetivo: Avaliar a segurança e a eficácia do tratamento tópico de feridas com células estromais mesenquimais derivadas da medula óssea cultivadas e suspensas em lisado plaquetário. **Método:** Estudo prospectivo intervencionista e controlado com 42 camundongos C57BL/6 com ferida excisional induzida. Eles foram divididos em quatro grupos de tratamento: hidrogel (tratamento padrão de feridas), gel salino (controle), gel de lisado de plaquetas e gel de lisado de plaquetas contendo as células estromais mesenquimais. As análises foram feitas no dia 7 (n=5) e no dia 13 (n=7) para imuno-histoquímica, e no dia 21 (n=30) para segurança. O acompanhamento ocorreu por meio de avaliação clínica (eventos adversos, peso, dor, comportamento, redução do tamanho da ferida) e histológica (cicatrização, vascularização, colagenização). **Resultados:** Em

[1]Hospital de Clínicas de Porto Alegre – Porto Alegre (RS), Brazil.
[2]Universidade Federal do Rio Grande do Sul, School of Medicine, Graduate Program in Medical Sciences – Porto Alegre (RS), Brazil.
[3]Universidade Federal do Rio Grande do Sul, School of Dentistry – Porto Alegre (RS), Brazil.
[4]Universidade Federal do Rio Grande do Sul, Instituto de Ciências Básicas da Saúde – Porto Alegre (RS), Brazil.
*Corresponding author: lsilla@hcpa.edu.br

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relação à segurança, não foram identificados eventos adversos ou alterações fisiológicas relacionados ao tratamento. O grupo tratado com células apresentou melhores resultados na cicatrização através das avaliações macro e microscópicas, seguido pelo grupo gel de lisado plaquetário. **Conclusão:** A aplicação tópica de células estromais mesenquimais foi segura e mostrou resultados favoráveis.

DESCRITORES: Cicatrização de feridas. Célula estromal mesenquimal. Terapia celular. Lisado plaquetário. Estomaterapia.

Células estromales mesenquimales suspendidas en un gel de lisado plaquetario mejoran la cicatrización de heridas en ratones

RESUMEN

Objetivo: Evaluar la seguridad y la eficacia del tratamiento tópico de heridas con células estromales mesenquimales derivadas de médula ósea cultivadas y suspendidas en lisado plaquetario. **Método:** Estudio prospectivo, intervencionista y controlado con 42 ratones C57BL/6 con una herida por escisión inducida. Fueron divididos en cuatro grupos de tratamiento: hidrogel (tratamiento estándar para heridas), gel salino (control), gel de lisado plaquetario y gel de lisado plaquetario que contenía células estromales mesenquimales. Los análisis se realizaron el día 7 (n=5) y el día 13 (n=7) para inmunohistoquímica, y el día 21 (n=30) para seguridad. El seguimiento se llevó a cabo mediante evaluación clínica (eventos adversos, peso, dolor, comportamiento y reducción del tamaño de la herida) e histológica (cicatrización, vascularización y colagenización). **Resultados:** En relación con la seguridad, no se identificaron eventos adversos ni alteraciones fisiológicas relacionadas con el tratamiento. El grupo tratado con células presentó mejores resultados de cicatrización en las evaluaciones macro y microscópicas, seguido por el grupo tratado con gel de lisado plaquetario. **Conclusión:** La aplicación tópica de células estromales mesenquimales fue segura y mostró resultados favorables.

DESCRIPTORES: Cicatrización de Heridas. Célula Estromal Mesenquimatosa. Terapia Celular. Lisado de Plaquetas. Estomaterapia.

INTRODUCTION

Wounds are defined as the disruption of any tissue or cellular integrity due to mechanical, physical, or metabolic injuries[1]. Several therapies seek to optimize healing time; however, some cases are difficult to heal or do not respond to any available treatment. In this context, mesenchymal stromal cells (MSCs) have emerged as potential candidates for the healing of acute and chronic wounds, regardless of etiology. Studies have demonstrated the efficacy and safety of MSCs in the treatment of burns,[2] diabetic foot ulcers,[3] and pressure injuries, both in preclinical and clinical settings,[4] helping to restore the tissue microenvironment.[5] The anti-inflammatory effect orchestrated by MSCs is due to their ability to migrate to sites of inflammation and secrete bioactive molecules, such as cytokines and growth factors, influencing other cell types and polarizing macrophages toward either a pro-inflammatory or anti-inflammatory phenotype[6].

MSCs can be applied to wounds in different ways, such as intramuscular or intradermal injections. However, complications have been reported, since the intra- and perilesional environment is already damaged[7]. The successive injections required for homogeneous application throughout the wound area may cause significant microinjuries in patients with impaired healing[8]. The safest route of administration appears to be topical application, ideally in gel form, as the product remains in contact with the wound bed without running off[9].

Platelet lysate is a biological product rich in growth factors, with a diverse and high protein content associated with essential cellular processes such as proliferation, morphogenesis, differentiation, biosynthesis, adhesion, and metabolism.[10] In addition to being used as a substitute for fetal bovine serum, it may also provide benefits in wound healing, which warrants further investigation.[11] Generally prepared in liquid form, when gelatinized it can serve as a vehicle for cells, providing a matrix that maintains their viability.[12] The association between stem cells and platelet derivatives, such as platelet-rich plasma or platelet gel in wound care, has been shown to increase the regenerative capacity of cells.[13] There appears to be modulation of cellular energy metabolism, resulting in greater pro-angiogenic potential[14] and increased mitochondrial adenosine triphosphate production, thereby accelerating the wound closure process and improving the elasticity of newly regenerated skin.

This preclinical study aimed to assess the safety and efficacy of human bone marrow-derived MSCs cultured in platelet lysate and produced under Good Manufacturing Practice (GMP) conditions for future clinical trials on wound healing.

OBJECTIVES

To assess the safety of topical wound treatment using human bone marrow-derived MSCs cultured and suspended in a platelet lysate gel (through the assessment of adverse events, behavioral aspects, pain, and body weight). Secondarily, viability and efficacy were analyzed through wound healing assessment using the Pressure Ulcer Scale for Healing (PUSH), reduction in wound area size by measurement, angiogenesis by immunohistochemistry, and collagen fibers.

METHODS

This was an *in vivo*, prospective, controlled study conducted at the Animal Experimentation Unit of *Hospital de Clínicas de Porto Alegre* (HCPA), after approval by the institution's Animal Use Ethics Committee under protocol number 2023-0051 and by *Plataforma Brasil* under Certificate of Presentation for Ethical Consideration number 68762923.1.0000.5327. All ethical principles for animal experimentation were followed throughout the study, in accordance with local regulations (Brazilian National Council for the Control of Animal Experimentation). The Consolidated Standards of Reporting Trials guideline was used in the preparation of this project.

Sample

Forty-two male, isogenic, 8-week-old C57BL/6 mice (Mus musculus), with a mean body weight of 21 grams, were studied. Sample size was calculated considering an effect size of f=0.57, with healing rate as the outcome, according to a similar study, a significance level (p) of 0.05, and a statistical power of 80% (G*Power software, v3.1.9.7)[15].

The animals were housed individually in microisolators containing wood shavings, with controlled temperature, humidity, and light–dark cycle. Food and water were provided ad libitum. Body weight and behavioral aspects were monitored.

The animals were randomly assigned to the following groups:

Group 1 – Control: hydrogel (n=6);

Group 2 – Treatment: platelet lysate gel (n=13);

Group 3 – Treatment: platelet lysate gel containing MSCs (n=15);

Group 4 – Control: saline gel (n=8).

The study was divided into different segments: up to day 7 (n=5) and day 13 (n=7) for immunohistochemical assessment, and up to day 21 for the assessment of adverse events and histological analysis (n=30, with 10 animals in each group).

Anesthesia, wound induction, and analgesia

The animals underwent general inhalation anesthesia using isoflurane vaporized in 500 mL of oxygen (5% for induction and 2–3% for maintenance) through a face mask. Under anesthesia, tramadol 20 mg·kg^{-1} was administered intraperitoneally, followed by antisepsis of the dorsal region with a 2% alcoholic chlorhexidine solution. An experimental excisional wound was induced by marking the wound area with a 6-mm circular template on the dorsal region between the scapulae. The marked skin was removed, producing a full-thickness wound (epidermis, dermis, and hypodermis) extending to the external surface of the muscular fascia. During the postoperative period, for three days, the animals received tramadol hydrochloride (20 $mg{\cdot}kg^{-1}$) intraperitoneally every 12 hours for pain control. Pain assessment was performed using the Grimace Scale[16].

Treatment

The hydrogel is a product locally manufactured by the HCPA industrial pharmacy and consists of 77.7% water, 20% propylene glycol, and 2.3% carboxymethylcellulose. The saline gel is based on 0.9% sodium chloride and uses the same pharmaceutical gelling agent employed in the platelet lysate gel. The exact formulation of the products (saline gel and platelet lysate gel) is currently confidential, as recommended by the institution's Innovation and Technology Transfer Center.

Platelet lysate was produced from pooled human platelet bags discarded by the blood bank due to expiration of the storage period and after negative serological testing for infectious diseases. The production method has been previously described[17]. In summary, platelets underwent freeze–thaw cycles for activation, followed by centrifugation and filtration, heparin addition, and subsequent conversion into a gel through the addition of a gelling agent.

After obtaining written informed consent, MSCs were obtained by washing bags and filters that would otherwise be discarded after bone marrow transplantation procedures from healthy donors. The cells were plated in Dulbecco's Modified Eagle Medium (Gibco) supplemented with platelet lysate until reaching 80% confluence and underwent three trypsinization passages (P0, P1, and P2).[18] They were cryopreserved at -80°C while quality control testing was performed (flow cytometry, mycoplasma testing, bacteriological detection, and endotoxin testing). They were characterized as >97% CD90+, CD75+, CD105+; ≤3% CD14+, CD34+, CD19+; and ≤3% CD45+, CD3+, HLA-DR+.[19] At the time of use, the cells were thawed and resuspended in platelet gel at a concentration of 2×10^6 cells/cm^2 of wound area.[20] To assess cell viability in the platelet lysate gel, two methods were used: Trypan Blue and the Tetrazolium Dye (MTT) assay.

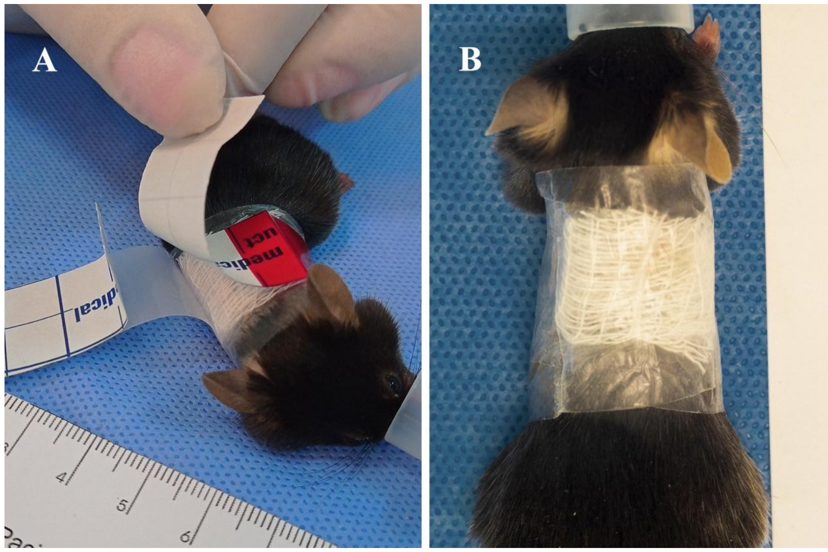
Dressing

After topical application of the treatment, the wounds were protected with a multilayer dressing to comply with wound-healing principles, preserving the wound from contaminants and trauma. The dressing was adapted from the model proposed by Amorim.[21] As the primary dressing, a silicone adhesive mesh (Cuticell® Contact) measuring 1.5 × 2 cm was used, followed by a sterile cotton gauze pad (Hérika®) of the same size and secured with two transparent adhesive polyurethane films (Hypafix®) measuring 3 × 8 cm. The first film was applied in the dorsoventral direction and the second in the opposite direction, wrapping twice around the animal's body (Figure 1).

Treatment application and dressing changes were performed on days 0, 3, 6, and 9 post-surgery, with the animals under light sedation. On day 12, the dressings were removed because the wounds had practically healed. During dressing changes, the wounds were cleaned with a sterile cotton swab soaked in 0.9% saline solution. In case of scabs, these were gently removed so that treatments could be reapplied directly to the wound.

Clinical assessment of wounds

Macroscopic assessment was performed on days 0, 3, 6, 9, 12, 15, and 21 after wound excision, with the animals under brief anesthesia. Clinical aspects such as pain, pruritus, excoriation, odor, infection, hyperemia, edema, crust formation, hematoma, amount and appearance of exudate (serous, serosanguineous, bloody, and purulent), and tissue type (necrotic, slough, granulation, or epithelialization) were observed. Wound assessment was also performed using the PUSH Tool (U.S. National Pressure Injury Advisory Panel, Schaumburg, Illinois, USA), validated for human wounds of different etiologies. The wounds were photographed using a Sony® Alpha 7 digital camera with ISO 400 settings, a Sigma® 50 mm macro lens with the aperture set at f/8.0, using only ambient artificial lighting and a transparent acrylic support, maintaining the same distance in all photographs. Wound area was measured using ImageJ® software (U. S. National Institutes of Health, Bethesda, Maryland, USA).



Source: Prepared by the authors.

Figure 1. Multilayer dressing. **(A)** Application of the transparent film in the ventral-to-dorsal direction, finishing over the other layers; **(B)** Final appearance of the secured dressing.

Sample collection and euthanasia

With the animals under deep isoflurane anesthesia, skin fragments containing the wound area and a margin of healthy skin were removed. Euthanasia was performed by exsanguination under deep anesthesia and intracardiac puncture; the heart, lungs, spleen, kidneys, and liver were removed for future analysis. The collected samples were immediately placed in 10% buffered formaldehyde and processed using the institution's routine paraffin-embedding technique.

Microscopic assessment of wounds

The microscopic healing parameters analyzed were inflammatory infiltration, tissue type, collagenization, and vascularization. The pathologist was blinded to the treatments and used a Nikon® Eclipse Si microscope with 10× magnification. For histological assessment, sections were stained with hematoxylin and eosin. Inflammatory infiltration was assessed according to its density, and a score ranging from 0 to 3 was assigned: 0 for absence of inflammatory infiltration, 1 for mild infiltration, 2 for moderate infiltration, and 3 for severe infiltration. Regarding tissue type, a score of 0 was assigned when no alterations could be identified compared with the remaining section (indicating complete healing), and a score of 1 when granulation tissue was identified.

Collagen fibers were assessed using Masson's Trichrome staining. Five images were obtained from each slide, and the area was analyzed using ImageJ® software with the "color deconvolution" plugin. Collagenization was quantified as the mean area of the five fields. To assess angiogenesis, CD31 immunohistochemistry was used with 20× magnification. Five images were captured, and blood vessels were counted, totaling the vessels present in each sample.

Statistics

Statistical analysis was performed using SPSS software (SPSS Inc. Released 2009. PASW Statistics for Windows, Version 18.0. Chicago: SPSS Inc.). The distribution of variables regarding normality was assessed using the Shapiro-Wilk test and the Q-Q plot. Categorical variables, such as inflammatory cells, tissue type, and edema, were compared among groups using Fisher's exact test.

To compare characteristics over time among groups, the Generalized Estimating Equations model was used, with binomial distribution and logit link function for dichotomous categorical variables, such as the presence of crusts. For continuous variables such as wound area measurement, degree of excoriation, PUSH score, and exudate type, the same Generalized Estimating Equations analysis was used, with normal distribution and identity link function.

A p-value lower than 0.05 was considered statistically significant.

RESULTS

Two animals were lost during the immediate postoperative period (one due to hemorrhage and the other due to weight loss exceeding the final limit established by the animal ethics committee [<10%]), and were excluded from the study by isoflurane overdose.

Cell viability in the platelet lysate gel was verified using the MTT colorimetric assay and the Trypan Blue method through culture in an incubator (37 °C, 5% CO_2) for different periods. After 24 hours, 56% of the cells remained viable; after 48 hours, 26%; and after 72 hours, 11.5%. Experiments were performed in triplicate for each method.

The dressings remained covering the wounds 97.47% of the time; only four animals removed them on a single occasion each. The animals showed constant attention to the presence of the dressings, persistently attempting to remove them. When abrasions were identified, they were treated with 2% iodine tincture only at the affected site.

Comparing the groups, there was no difference in weight loss (p=0.118), only weight variation over time within each group ($p < 0.001$), as the animals regained weight on different days until reaching their initial weight (Table 1). Animals in Group 3 regained weight much earlier than those in the other groups.

The animals were assessed for behavioral and clinical aspects by the same non-blinded evaluator. No treatment-related changes were observed in terms of behavior or signs of pain. There were no serious adverse events, such as functional impairment or death. The organs showed no macroscopic alterations during postmortem inspection. No wound alterations were observed, such as necrosis, dehiscence, hematoma, hyperchromia, or signs of infection, including the presence of purulent content, hyperemia, or irritative conditions such as perilesional dermatitis, in any of the groups. Mild edema at the wound edges was identified on the third postoperative day in all groups and disappeared in the following days. Draining exudate was absent during most of the study period (60.5%) and was more frequent during the first days, decreasing until day 12. The most frequently observed type was serous exudate (26.3%), followed by bloody (10.5%) and serosanguineous (2.6%) exudate. The occurrence of exudation was similar among the groups. Crust formation was observed from day 3 to day 15 of the experiment in 30% of the animals across all groups.

Immediately after the surgical procedure, the excised areas did not differ among the groups (p=0.508). On day 3, there was a 6.9% increase in area (mean of 0.31 mm) in Groups 1, 2, and 3, whereas a reduction was observed in Group 4. On subsequent days, all groups presented the same mean wound size until wound closure (day 12), epithelialization (day 15), and hair regrowth (day 21) (Figure 2). There was a difference among the groups regarding wound size over time (p=0.001), because Group 3 showed slightly slower wound closure than the others, whereas Group 4 closed after the remaining groups.

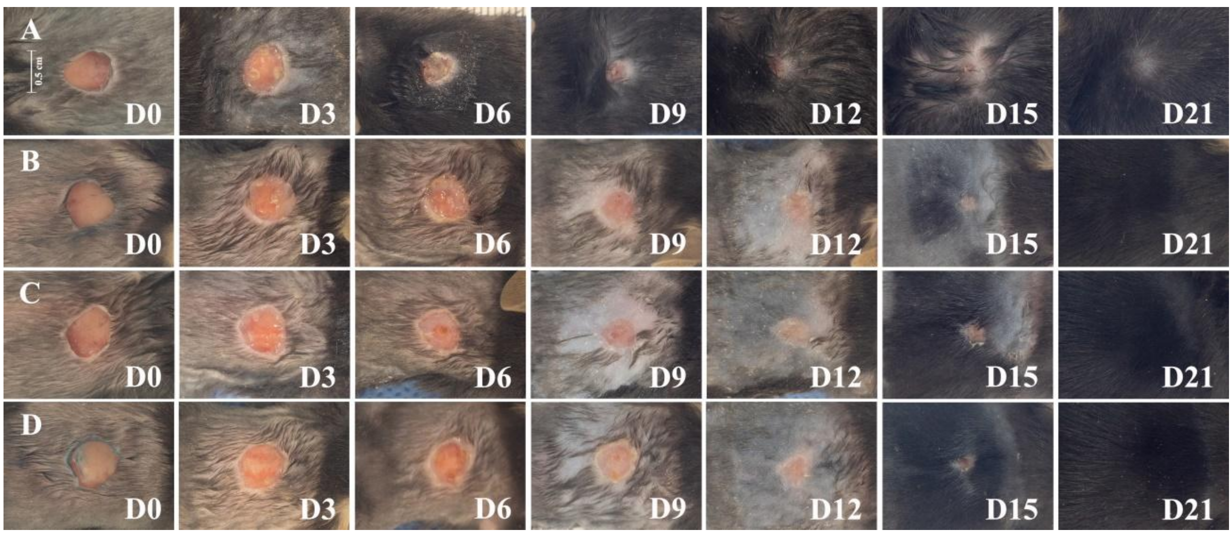
Table 1. Animal body weight. Comparison between the animals' mean initial body weight by group before the experiment (d−1) and body weight after the procedure (d+1), with the respective percentages of weight loss and the time required to recover the initial body weight.

Group	Mean weight (d-1)	Mean weight (d+1)	Weight loss (%)	Time to recover initial weight (days)
1. Hydrogel	22.45 (SD 2.57)	21.69 (SD 2.57)	3.39%	15
2. Platelet lysate gel	20.83 (SD 2.30)	19.80 (SD 1.90)	4.4%	15
3. Platelet lysate gel + MSCs	20.92 (SD 2.10)	20.40 (SD 1.86)	2.49%	3
4. Saline gel	19.83 (SD 1.54)	19.37 (SD 1.66)	2.32%	9

Weight in grams.
d: days; SD: standard deviation.
Source: Prepared by the authors.

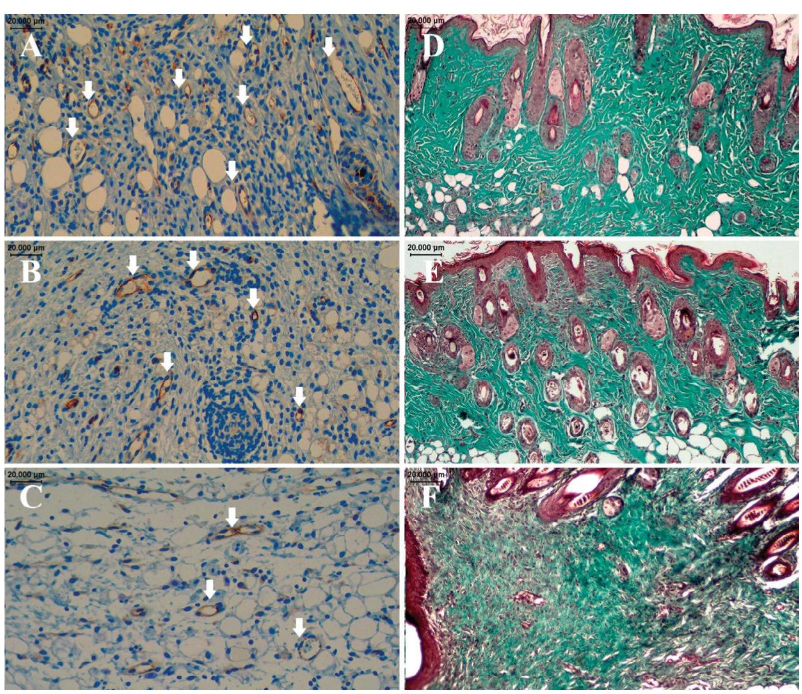
The PUSH score is calculated as the sum of three parameters: wound area (score 0–10), amount of exudate (0–3), and wound bed appearance (0–4). The maximum score achieved was 6 points. Scores decreased over time in all groups, indicating healing. Treatment Groups 2 and 3 showed slightly lower scores over time, indicating a trend toward better healing parameters (p=0.027).

Similar collagenization was observed among the groups, with 196 collagen fibers in Group 1 (mean, SD 3.0), 191 in Group 2 (SD 9.4), 186 in Group 4 (SD 4.8), and 183 in Group 3 (SD 4.8) (Figure 3).



Source: Prepared by the authors.

Figure 2. Healing over time. Evolution of the wounds, representative of each group throughout the study period (D=days). **(A)** Group 1 – Hydrogel; (B) Group 2 – Platelet lysate gel; **(C)** Group 3 – Mesenchymal stromal cells + platelet lysate gel; **(D)** Saline gel.



Source: Prepared by the authors.

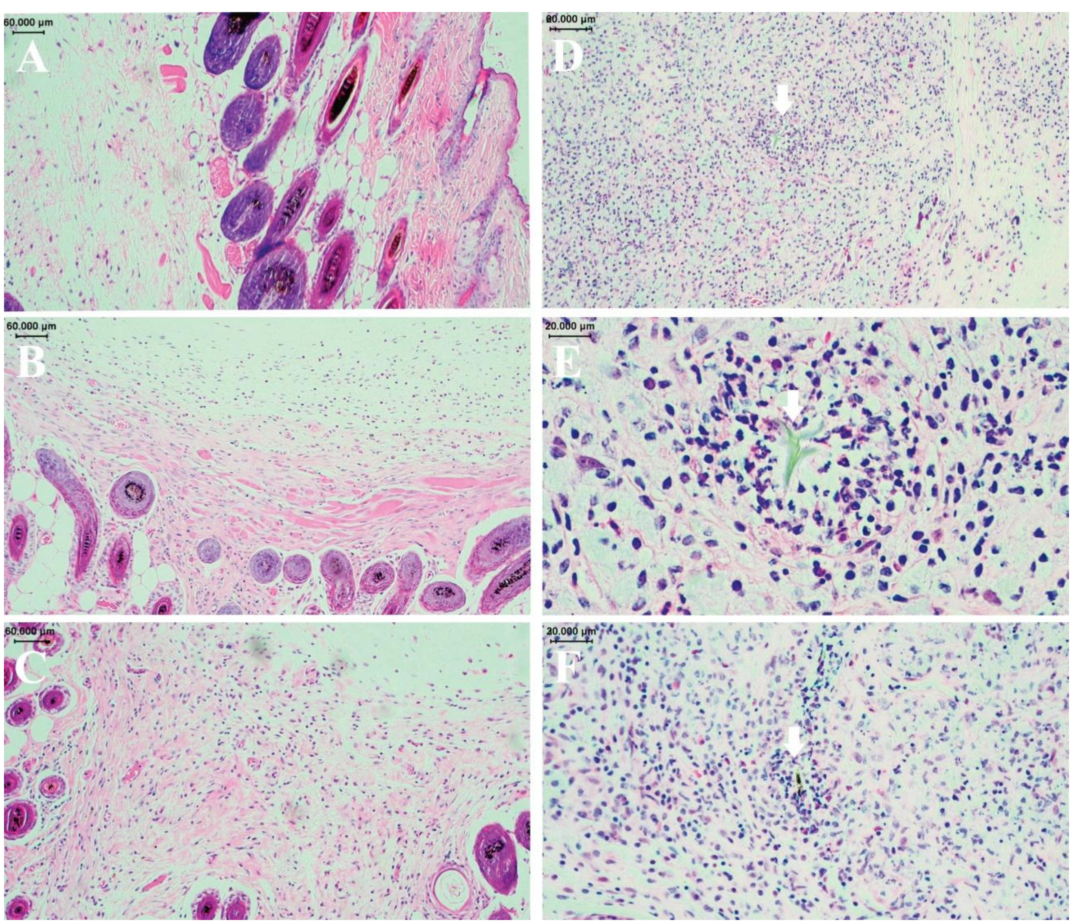
Figure 3. Microscopic assessments. In the first column, CD31 staining, with blood vessels identified by arrows in Group 3 **(A)**, Group 2 **(B)**, and Group 1 **(C)**; in the second column, collagen stained with Masson's Trichrome in Group 3 **(D)**, Group 2 **(E)**, and Group 1 **(F)**.

When angiogenesis was assessed, Group 3 showed a greater number of vessels (mean 51.6, SD 11.6), followed by Group 2 (46, SD 5.6), compared with the mean values observed in Control Groups 1 and 4 (41.6, SD 0.7). However, this result did not reach statistical significance (p=0.493) (Figure 3).

Groups 2 and 3 showed a similar pattern of mild inflammatory infiltrate, with mean scores of 0.9 (SD 0.7) and 1.0 (SD 0.8), respectively. Control Groups 1 and 4 showed opposite results: severe inflammation in Group 1 (3 points, SD 0), the highest among all groups ($p < 0.001$), and the lowest degree of inflammation in Group 4 (mean 0.20, SD 0.4). Granulation tissue was present in 20.7% of the animals, with Groups 1 and 3 showing rates of 100% and 20%, respectively, unlike the other groups ($p < 0.001$) (Figure 4).

DISCUSSION

This study assessed the safety of the topical use of MSCs suspended in a platelet lysate gel for the treatment of wounds in mice. No treatment-related adverse events were identified. Physiological changes, such as the presence of edema at the wound edges during the first postoperative days, as well as exudate drainage and crust formation, occurred equally among the groups. These findings are common during the inflammatory phase, in which tissue damage acts as the initial stimulus for healing, bringing blood components into contact with collagen and other extracellular matrix substances, thereby inducing platelet degranulation and activation of the coagulation cascade, with the release of vasoactive mediators (such as histamine, serotonin, and bradykinin) and chemotactic agents. Subsequently, inflammatory cells are recruited to the



Source: Prepared by the authors.

Figure 4. Inflammatory infiltrate. Hematoxylin and eosin staining showing absence of infiltrate **(A)**, mild infiltrate **(B)**, moderate infiltrate **(C)**, severe infiltrate **(D)** with granulation tissue (arrow), and granulation tissue (arrow) in **(E)** and **(F)**.

wound, causing heat, redness, and edema, the latter resulting from increased capillary permeability and consequent fluid leakage into the extracellular space.

The animal model selected for this study was the C57BL/6 mouse, the most widely studied model in stem cell biomedical research.[22] This strain also exhibits genetic uniformity, ensuring a consistent physiological response. Regarding the dressing used, it remained in place throughout the study, which is challenging when working with small and active rodents. The dressing ensured treatment retention and maintained protection of the wound area.

Body weight is an important predictor of animal health. All mice lost weight similarly during the postoperative period (p=0.118), which was expected. The wound-induction surgery was moderately invasive, causing extensive skin ulceration, repeated handling, anesthesia exposure, surgical pain (despite analgesic treatment), and discomfort related to dressing changes. The time required to regain initial body weight differed among groups ($p < 0.001$), as the cell-treated group required only three days, likely because postoperative recovery was facilitated by MSCs through reduced energy expenditure, given that wound healing is a highly metabolically demanding process[5].

All groups achieved complete wound healing before the end of the observation period (day 21). Wounds treated with cells exhibited slightly slower closure, which may be explained by an enhancement of the healing process. This group showed a shorter postoperative weight recovery period, slightly better healing rates according to the PUSH scale, a mild inflammatory profile, and a tendency toward better vascularization than the other groups. Platelet lysate achieved the best mean score on the aforementioned scale over time and also demonstrated a mild inflammatory profile. Saline gel showed the lowest inflammatory profile and was the only group to exhibit wound-size reduction on postoperative day 1; however, it did not heal earlier than the other groups and presented the third-best mean PUSH score. Hydrogel showed the highest inflammatory profile and the poorest healing indices, tending to present the lowest vascularization and the greatest amount of residual microscopic granulation tissue.

Although the cell-treated group, followed by the platelet lysate group, exhibited a greater number of blood vessels in the wound, this result could not be statistically confirmed because of the small sample size. This study hypothesizes that these treatment groups presented a higher vessel count, in agreement with the literature, which attributes this effect to a greater presence of vascular endothelial growth factor, inducing neoangiogenesis.[23] Safety analysis was prioritized over treatment efficacy to avoid sacrificing additional animals. Our preliminary results are consistent with the existing literature on the subject.

Microscopically, granulation tissue was identified in wound tissue fragments from animals in Group 1 (100%) and Group 3 (20%) on day 21. The persistence of granulation tissue in Group 1 may be explained by its poorer healing performance and higher inflammatory profile, which microscopically could be interpreted as delayed healing compared with the other groups, since granulation tissue tends to regress as inflammatory infiltrates resolve. Group 3 also presented granulation tissue, probably because its healing rate was slightly slower than that of the other groups, possibly reflecting a more elaborate healing process that generates abundant granulation tissue still undergoing degradation.

The number of collagen fibers among the groups was considered similar; however, differences related to collagen fiber quality may exist and were not assessed. The dermis contains approximately 80% type I collagen and 20% type III collagen, whereas granulation tissue may contain up to 40% type III collagen, which is considered immature. Therefore, it is hypothesized that the groups with better healing outcomes may have developed superior collagen fiber characteristics.

Regarding inflammatory infiltrate, the highest index was observed in the hydrogel group, whereas the cell and platelet lysate groups showed mild inflammation. The lowest index was observed in the saline gel group. Inflammation is a fundamental component of the healing process and may result in impaired or delayed healing when either excessive or insufficient. The low inflammatory profile observed with saline gel did not confer benefits, reinforcing the concept that a certain degree of inflammation is necessary for healing. Although MSCs are primarily recognized for their anti-inflammatory properties,[5] they maintain the microenvironment with low levels of interleukin-1 and tumor necrosis factor-alpha, both pro-inflammatory mediators. Therefore, they possess immunomodulatory capacity, increasing inflammation when necessary and suppressing it when excessively present[24].

The wounds in this study were acute and had no underlying pathophysiology; therefore, a controlled inflammatory response was expected, and treatment consisted primarily of environmental management and infection prevention. In chronic wounds, which may have multiple etiologies, the underlying clinical condition should always be considered in addition to local management measures. Recovery barriers associated with underlying diseases and/or increased risk factors may lead to an unpredictable healing course, in which persistent inflammation must be controlled. The biological treatments presented in this study may be more appropriately investigated in hard-to-heal wounds.

The platelet lysate gel, in addition to maintaining cell viability when combined with mesenchymal cells, also confirmed its biological healing properties when used alone. It qualifies as a novel therapeutic possibility because it provides vesicles, growth factors, and other molecules known to be important for chemotaxis.[10] MSCs confirmed their wound-healing properties, tending toward the best outcomes in the study. All treatments were prepared according to GMP guidelines used for manipulated biological products, with source traceability and safe-origin materials, as required by national legislation established by Resolution of the Collegiate Board 508 of the Brazilian Health Regulatory Agency[25].

The platelet lysate gel, with or without cells, is aligned with recent research in bioengineering, cell therapy, and wound care, which recommends the use of biomaterials that serve as scaffolds for cells, generating three-dimensional matrices. It is important to combine mesenchymal cells with other regenerative therapies, providing a vehicle for the transport of soluble healing factors through a structure resembling the natural extracellular matrix, with biocompatible, bioactive, and biodegradable properties when releasing products into biological systems. Therefore, the three products developed for this study showed distinct therapeutic outcomes and achieved their intended purpose. To the best of our knowledge, this is the first study to assess the topical application of bone marrow-derived MSCs cultured and suspended in platelet lysate for wound treatment.

Study limitations

Although the cell-treated group, followed by the platelet lysate group, presented a greater number of blood vessels in the wound, this result could not be statistically confirmed because of the small sample size. The CD31 sample was not expanded to avoid causing additional harm to animals for an outcome with an already established scientific basis. Safety analysis (which extended until day 21) was prioritized. The number of collagen fibers among the groups was considered similar; however, there may be differences related to fiber quality that could not be assessed.

Recommendations

The results suggested that the combination of mesenchymal cells with platelet lysate gel achieved better efficacy outcomes in wound healing, followed by platelet lysate gel alone. Both should continue to be investigated as therapeutic alternatives to assist patients with hard-to-heal wounds.

CONCLUSION

Human bone marrow-derived mesenchymal stem cells produced under GMP conditions were safe when applied topically to wounds in mice, with no adverse events beyond the expected secondary effects of the selected wound model. The results suggested that the combination of mesenchymal cells with platelet lysate gel achieved better efficacy outcomes in wound healing, followed by platelet lysate gel alone. Both should continue to be investigated as therapeutic alternatives to assist patients with hard-to-heal wounds.

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