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BIOINNOVATIVE STRATEGY FOR BURN HEALING: NANOCELLULOSE AND GRAPHENE OXIDE MEMBRANE ASSOCIATED WITH VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF)

ESTRATÉGIA BIOINOVADORA PARA A CICATRIZAÇÃO DE QUEIMADURAS: MEMBRANA DE NANOCELULOSE E ÓXIDO DE GRAFENO ASSOCIADA AO FATOR DE CRESCIMENTO ENDOTELIAL VASCULAR (VEGF)

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Image



Fixing platform for burn induction device

Central Message

The high prevalence of burns represents an important challenge for public health, both because of the high cost of its treatment and because of the continuous need to develop ideal, low-cost dressings capable of promoting faster and more effective

healing. Thus, experimental studies with new ideas are opportune for the creation of new devices with greater efficiency.

Perspective

The nanocellulose membrane enriched with graphene oxide and VEGF has been shown to accelerate and improve burn healing in rats, favoring the adequate progression of the reparatory phases, with inflammatory modulation, angiogenic stimulation and better tissue organization. Its superior performance to control suggests translational potential as an advanced dressing, although additional studies are needed to elucidate mechanisms, safety, and clinical applicability.

Declaration of availability of research data

This manuscript is an original article and, therefore, did not generate primary data. All referenced data derive exclusively from previously published and publicly available articles in scientific databases, including SciELO, PubMed, Scopus, and Google Scholar. No original datasets, experimental spreadsheets, laboratory images, or any type of primary data that requires deposit in an open access repository were produced. To comply with the SciELO Preprints Open Science guidelines, we declare that there is no data to be made available other than the cited references themselves, which are publicly accessible. Thus, the research is based entirely on secondary information obtained from the scientific literature already published, and on the new data from this research that can be shared.

Authors' contributions

Nerlan Tadeu Gonçalves de Carvalho - Conceptualization

Fernando Issamu Tabushi - Methodology

Aryel Heitor Ferreira - Methodology

Eduarda Cesario Bottini Bastos – Conceptualization, Validation

Raiane Alvarenga Ranieri - Conceptualization

Carolina Kleemann - Conceptualization

Oswaldo Malafaia – Project Administration, Writing (proofreading and editing)

ABSTRACT – Introduction: The high prevalence of burns represents an important challenge for public health, both due to the high cost of its treatment and the continuous need to develop ideal, low-cost dressings capable of promoting faster and more effective healing. **Objective:** To evaluate the nanocellulose membrane enriched with graphene oxide and vascular endothelial growth factor in the healing of burns in rat skin. **Method:** A total of 36 adult male Wistar rats (*Rattus norvegicus albinus*) were divided into a control group (C) and an experimental group (E). Each group was subdivided into two subgroups according to the dates of sacrifice (seven and 14 days). The parameters evaluated were macroscopy, wound contraction rate and histological aspects such as neutrophilic exudate, lymphoplasmacytic infiltrate, quantification of capillaries, fibroblasts and collagen fibers, scar depth and wound re-epithelialization. **Result:** At seven days, the rate of wound contraction did not differ between the groups, and at 14 days both showed similar progression over time. Angiogenesis showed slight superiority in the experimental group on the 7th day, becoming equivalent between the groups on the 14th day. Neutrophilic exudate was more evident in the control group in the initial period, while the experimental group showed a predominance of mild inflammatory response, a pattern that was maintained at 14 days. Lymphoplasmacytic infiltrate showed similar behavior between the groups in both periods evaluated. The

fibroblasts amount was initially low in both groups, increasing comparably at 14 days. As for collagen, the experimental group showed greater fiber maturation in the late period, unlike the control, which remained with a predominance of immature fibers. Scar depth was greater in the control group on the seventh day, while the experimental group exhibited lesions more evenly distributed between superficial, intermediate, and deep. **Conclusion:** The nanocellulose membrane enriched with graphene oxide and VEGF has been shown to accelerate and improve burn healing in rats, favoring the adequate progression of the repair phases, with inflammatory modulation, angiogenic stimulation and better tissue organization. Its superior performance to control suggests translational potential as an advanced dressing, although additional studies are needed to elucidate mechanisms, safety, and clinical applicability.

KEYWORDS - Burns. Healing. Nanocellulose. Graphene. Vascular endothelial growth factor.

RESUMO – Introdução: A elevada prevalência de queimaduras representa importante desafio para a saúde pública, tanto pelo elevado custo de seu tratamento quanto pela contínua necessidade de desenvolver curativos ideais, de baixo custo e capazes de promover cicatrização mais rápida e eficaz. **Objetivo:** Avaliar a membrana de nanocelulose enriquecida com óxido de grafeno e fator de crescimento endotelial vascular na cicatrização de queimaduras em pele de ratos. **Método:** Foram utilizados 36 ratos Wistar (*Rattus norvegicus albinus*) machos, adultos que foram divididos em grupo controle (C) e grupo experimento (E). Cada grupo foi subdividido em dois subgrupos conforme as datas de sacrifício (sete e 14 dias). Os parâmetros avaliados foram a macroscopia, taxa de contração das feridas e aspectos histológicos como exsudato neutrofílico, infiltrado linfoplasmocitário, quantificação de capilares, fibroblastos e fibras colágenas, profundidade da cicatriz e reepitelização das feridas. **Resultado:** No período de sete dias, a taxa de contração das feridas não diferiu entre os grupos, e aos 14 dias ambos apresentaram progressão semelhante ao longo do tempo. A angiogênese mostrou discreta superioridade do grupo experimental no sétimo dia, tornando-se equivalente entre os grupos no 14º. O exsudato neutrofílico foi mais evidente no grupo controle no período inicial, enquanto o grupo experimental apresentou predomínio de resposta inflamatória leve, padrão que se manteve aos 14 dias. O infiltrado linfoplasmocitário mostrou comportamento semelhante entre os grupos em ambos os períodos avaliados. A quantidade de fibroblastos foi inicialmente baixa nos dois grupos, aumentando de forma comparável aos 14 dias. Quanto ao colágeno, o grupo experimental apresentou maior maturação das fibras no período tardio, diferentemente do controle, que permaneceu com predominância de fibras imaturas. A profundidade da cicatriz foi maior no grupo controle no sétimo dia, enquanto o grupo experimental exibiu lesões mais uniformemente distribuídas entre superficiais, intermediárias e profundas. **Conclusão:** A membrana de nanocelulose enriquecida com óxido de grafeno e VEGF demonstrou acelerar e aprimorar a cicatrização de queimaduras em ratos, favorecendo a progressão adequada das fases reparatórias, com modulação inflamatória, estímulo angiogênico e melhor organização tecidual. Seu desempenho superior ao controle sugere potencial translacional como curativo avançado, embora estudos adicionais sejam necessários para elucidar mecanismos, segurança e aplicabilidade clínica.

PALAVRAS-CHAVE - Queimaduras. Cicatrização. Nanocelulose. Grafeno. Fator de crescimento endotelial vascular.

INTRODUCTION

Burns are injuries caused by various agents that promote the destruction of the skin, which can lead to the exposure of deeper tissues. According to the World Health Organization (WHO), about one million accidents occur in Brazil per year, of which 100 thousand resort to hospital care, resulting in 3,000 deaths.¹ This significant number is associated with the high cost of home or hospital treatment, time off work, and physical and psychological sequelae.^{2,3}

Burns can be classified according to the type of causal agent into thermal, electrical, chemical, radioactivity, and abrasion. Those resulting from thermal agents are the most common and become more serious when heated gases are inhaled, causing spasm, injury to the epithelium of the trachea and bronchi, extravasation of exudates and death by asphyxiation. Depending on the extent of the burn, fluid losses lead to hydroelectrolyte imbalance, which if not addressed correctly can cause shock and death.

The immediate analysis of those who suffered burns based on the extent, according to Wallace's Rule^{1,4} or the Lund and Browder⁵, establishes care when treating. The same criterion applies to evaluate the degree of burns, classifying them as first, second, third and fourth degrees. Considering its different types, care costs, and developments, it is important to encourage awareness campaigns among the population and the use of personal protective equipment (PPE) and, likewise, the need to create ideal dressings, at low cost and capable of promoting faster and more effective healing.

Thus, it is of great relevance to research new materials for treatment. Its different causes, immediate approach, care cost and consequences, are of high importance, the reason for the present work in the development of new products that make recovery faster, less painful and with fewer sequelae.

Thus, the aim of this study was to evaluate the nanocellulose membrane enriched with graphene oxide and vascular endothelial growth factor (VEGF) in the healing of burns in rat skin, and the impact of this expression in an experimental model on the healing of deep burns.

METHOD

The study was carried out in the vivarium of Evangelical Machenzie Faculty of Paraná – FEMPAR and in the Master and PhD degrees in Surgical Sciences of Principles of Surgery program, both in Curitiba, PR, Brazil, and the membranes were provided by the Mackenzie Institute for Research in Graphene and Nanotechnologies (MackGraphe), São Paulo, SP, Brazil.

All procedures were carried out in accordance with the ethical standards for the experimental study of animals provided for in Brazilian legislation (Arouca Law 11.794 Brazil, 2013), and only put into practice after a favorable opinion from the institution's Ethics Committee on the Use of Animals (CEUAs), protocol No. 0004/2025.

Sample, membranes and anesthetic procedure

A total of 36 Wistar rats (*Rattus norvegicus albinus*) males, adults aged between 100-130 days, weighing between 200-250 g, purchased from Anilab Animal's Laboratory and housed in the FEMPAR vivarium housed in appropriate cages (Figure A). They were distributed in two groups of 18 animals each, called control group (C) and experimental group (E). Each group was again subdivided into two subgroups, according to the day of sacrifice: seven-day and 14-day subgroup (Figure 1B).

The membranes studied, nanocellulose with glycerol incorporated with graphene oxide and vascular endothelial growth factor, were produced and supplied by the MackGrappe – Mackenzie Institute for Research in Graphene and Nanotechnologies, São Paulo, SP, Brazil

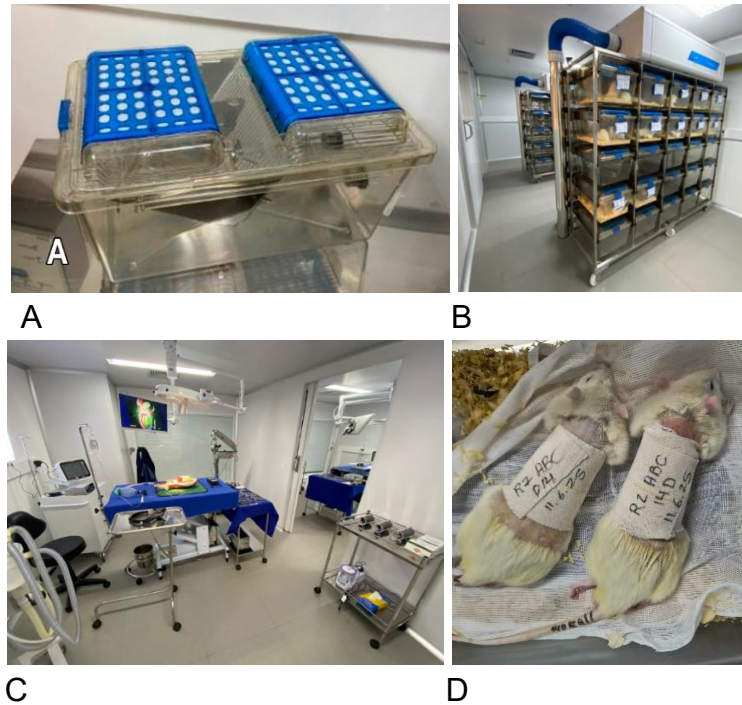
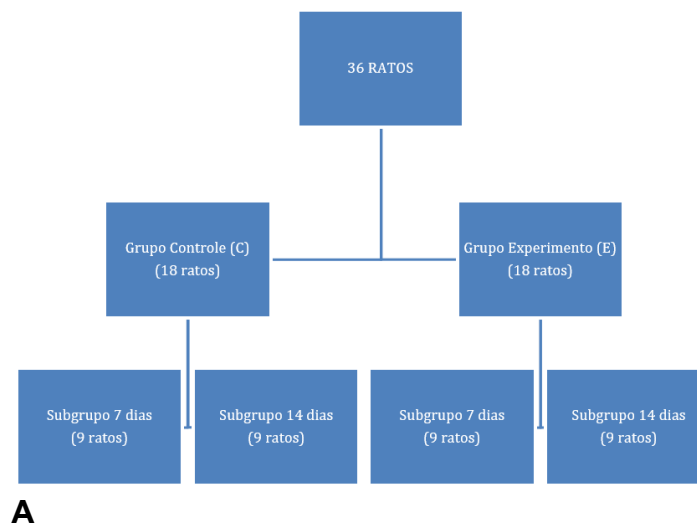


FIGURE 1 – A) Transparent polymer cage with perforated lid for accommodating guinea pigs; B) air-conditioned environment with stainless steel cabinet, containing cages arranged on shelves; C) operating room set up with X-ray machine, cardiac monitor and oximeter, anesthesia cart for inhalational anesthesia; D) animal wrapped in a thermal blanket during anesthetic recovery



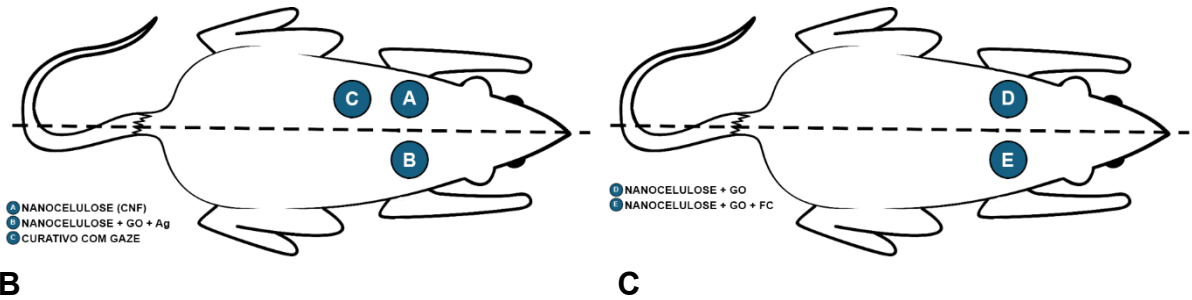


FIGURE 2 – A) Division of the groups; B and C) burn areas on the back of rats.

After the initial induction with isoflurane, the animals received general anesthesia intraperitoneally, using ketamine (Ketalar® – Aché) at a dose of 50 mg/kg associated with xylazine (Virbaxyl 2%® – Virbac) at a dose of 5 mg/kg. For analgesia, methadone was administered subcutaneously at a dose of 0.1 mg/kg. After the administration of the drugs, the animal was waited until the animal showed no response to handling and complete loss of mobility, criteria used to confirm the state of deep anesthesia.

Preparation of the surgical field and induction of burns

After anesthetization, the animals were submitted to trichotomy of the dorsal region, fixed with adhesive tape to the platform of the burn device and positioned in the supine position. All of them were induced to deep contact burns (Figures 2B and 2C) in the interscapular region by means of a device developed to produce heat from electrical energy that heats a copper extremity, circular in shape with a diameter of 1 cm (Figures 3A and 3B). The lesion was produced from the contact of the copper end heated to a temperature of 120 °C during a period of six seconds in the previously marked region. After the procedure, analgesics were administered.



FIGURE 3 – A) Base, device and temperature indicator used to produce the burn; B) tip that produces the burn; C) Fixation platform for the tip of the burn induction device; D) Flir Industrial reader

In order to standardize the burn, and eliminate possible bias caused by the researcher's pressure, the device was coupled to the platform, developed by the Stricto Sensu Graduate Program in Principles of Surgery at FEMPAR (Figure 3C), which,

when rested on the guinea pig's skin, exerted uniform and standard pressure on all tests, as well as the exposure time timed at six seconds (Figure 3A). To check the temperature established by the device, the Flir Industrial reader (Figure 3D) was used, which confirmed the temperature with a variation of 2-3 °C.

Clinical, analgesia, and postoperative care

After the surgical procedures, the animals were observed during anesthetic recovery, and were kept warm on thermal plates (Figure 1D). Analgesia was performed by administering tramadol hydrochloride (Tramadol®) at a dose of 5 mg/kg every 12 h during the first five days after burns and debridement.

During the entire period of permanence in the vivarium, the rats were kept in individual cages, under adequate conditions of hygiene, ventilation and lighting (Figure 1B).

After the anesthesia procedure and evaluation of the degree of sedation, the rats were placed in the prone position on the platform and the contact of the copper tip heated to 120 °C was made during the aforementioned six seconds in the previously demarcated region. Then, they were placed in a warm place and, after recovering from anesthesia, they received the analgesic.

Debridement of wounds, application of dressings and euthanasia

Forty-eight hours after burn induction, a new anesthesia (previously described) was performed for surgical debridement of the wound by excision of the necrotic area with a scalpel blade number 22 Feather®. After the excision of the devitalized tissue, the dressings were made with the membranes designated for each group, namely, dressing with gauze in the control group and with nanocellulose enriched with graphene oxide and vascular endothelial growth factor in the experimental group. The dressings were changed every five days, with the animals sedated with diazepam 5-10 mg/kg intramuscularly. After sedation, the wounds were cleaned with sterile distilled water and then each wound received, again, the specific treatment according to the group to which it belonged.

After the procedure, the animals were placed in their respective cages, containing one rat each, kept at room temperature, with an artificially performed light/dark cycle and with free access to water and CR-1 Nuvilab® feed. All cages were sanitized every 24 h (Figure 1B).

According to the schedule, after sedation with isoflurane, the animals were euthanized using 2 ml of the anesthetic solution (Sodium Thiopental® - Abbott Laboratories), at a concentration of 1 g diluted in 40 ml of saline solution injected into the peritoneal cavity. The entire experimental procedure, from wound making, dressing change and euthanasia, was performed in the operating room of the vivarium (Figures 1C and 1D).

Obtaining samples and evaluated parameters

After the animal's death was verified, verified through immobility and pupillary mydriasis, it was positioned in the prone position, without immobilization, and the measurement of the diameter of the wound was measured with a ruler graduated in centimeters, in addition to photographic records. With a number 20 scalpel blade, the previously demarcated skin was resected with about 1.5 cm from the edges of the lesions. The specimens obtained were deposited in flasks containing 10% formaldehyde solution to be sent for histological analysis.

Assessment of wound contraction rate

To evaluate this contraction, the animals were photographed on the day of debridement, at dressing changes every five days and on the day of euthanasia. Distance for photographic shots was standardized at 30 cm from the wound to the lens, through the use of a specific tripod. The photos were organized in a file for later analysis using the ImageJ software.

Histological evaluation

In the samples fixed in 10% formalin and in histological sections with a thickness of four micrometers, hematoxylin-eosin and Gomori trichrome stains were used. For the analysis, a 40X objective of the Olympus BX50 microscope was used, and quantitative evaluation of neutrophilic exudate, monomorphonuclear inflammatory infiltrate, fibroblasts, and neofomed capillaries were performed, all in three distinct high-magnification fields.

The analysis of collagen deposition from a quantitative and qualitative point of view was based on the amount of immature fibers (sanded fibers and in thin bundles) and mature fibers (thick fibers and in dense bundles). The following parameters were adopted: 0 = absent; 1 = with immature fibers; 2 = with a mixture of immature and mature fibers; and 3 = with mature fibers.

The epithelialization analysis was classified as initial when observed at the wound margin, intermediate when partially re-epithelialized, or total when fully re-epithelialized.

The analysis of the deep scar extension of the dermis was considered superficial when the process affected its upper third, intermediate when it reached the intermediate third, and deep when it reached the deep third of the dermis, which could reach the muscle aponeurosis.

Histologically, they were considered as 0 = absent, 1 = minimal; 2 = moderate and 3 = intense, the following indicators: neurophilic exudate, lymphoplasmacytic infiltrate, and fibroblasts. Capillaries were considered by their number in the field to be of great increase.

Statistical analysis

All data regarding burn contraction and number of capillaries were submitted to the Kolmogorov–Smirnov normality test and showed parametric distribution. Statistical analyses were then performed by one-way ANOVA, followed by Bonferroni post-test for multiple comparisons, when necessary.

RESULT

Macroscopic aspects of wounds

No macroscopic signs of secretion or infection were observed in any of the wounds evaluated, regardless of the group or subgroup (Figure 4).

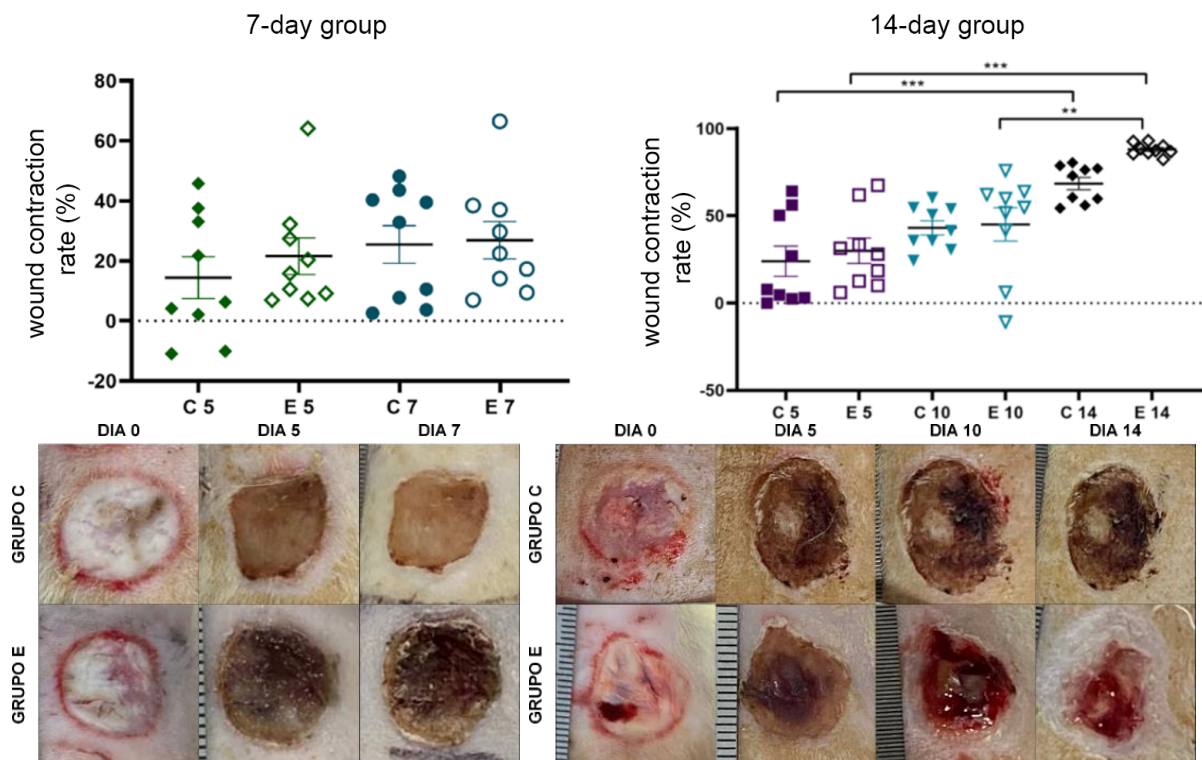


FIGURE 1 - Appearance of the wound on the 5th and 10th day of treatment with: A) gauze on the 5th day; B) nanocellulose membrane incorporated with graphene oxide and growth factor on the 5th day; C) gauze on the 10th day; D) nanocellulose membrane incorporated with graphene oxide and growth factor on the 10th day.

Quantitative comparison for the analysis of healing in groups C and E in relation to subgroups seven and 14 days

Contraction of wounds

When compared in the seven-day subgroup, no statistical difference was observed. However, the 14-day subgroup showed an increase in the rate of contraction over time, but there was no statistical difference between the groups (Figure 5).

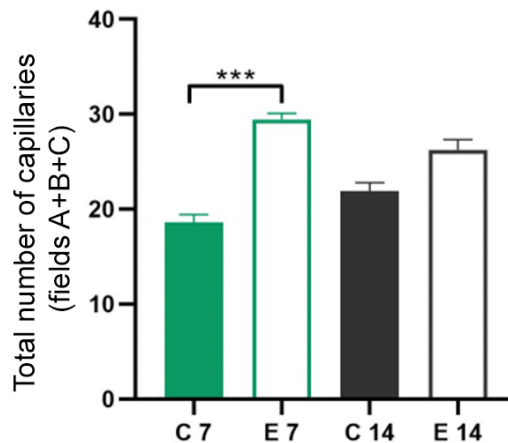


C = control group (dressing composed of sterile gauze); E = experiment group (dressing composed of nanocellulose, graphene oxide and VEGF factor) pointing to $p < 0.01$ and $p < 0.001$; Wound contraction rate was expressed as a percentage.

FIGURE 5 - Comparison of the contraction rate of C and E wounds in seven and 14 day subgroups

Total number of capillaries

Considering the sum of the three high-growth fields analyzed, an increase in the total number of capillaries was observed in group E at seven days compared to group C also at seven days. But on 14 days, the number was similar (Figure 6).

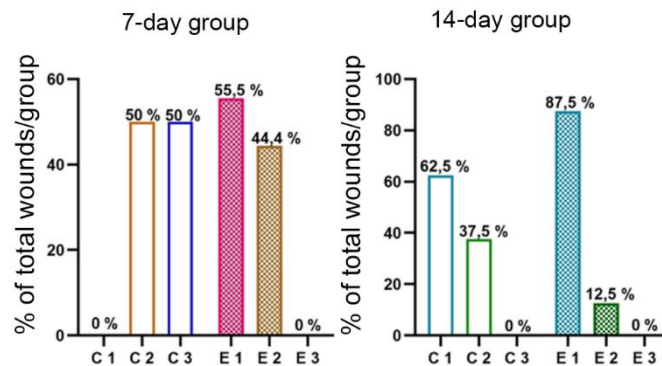


C = control group (dressing composed of sterile gauze); E = experimental group (dressing composed of nanocellulose, graphene oxide and VEGF factor) pointing to $p = 0.001$. Note: One-way ANOVA method, data expressed as mean \pm mean standard error (EPM)

FIGURE 6 - Comparison of the number of capillaries in seven and 14 day subgroups (three high-magnification fields)

Neutrophilic exudate

When comparing in percentages, it was observed that group C presented moderate or intense degrees, in the same percentage (C2, C3 = 50%); in E there was a predominance of the minimum degree (E1 = 55.5%, Figure 7). At 14 days, group E showed a predominance of minimal neutrophilic exudate (E1 = 87.5, Figure 7)

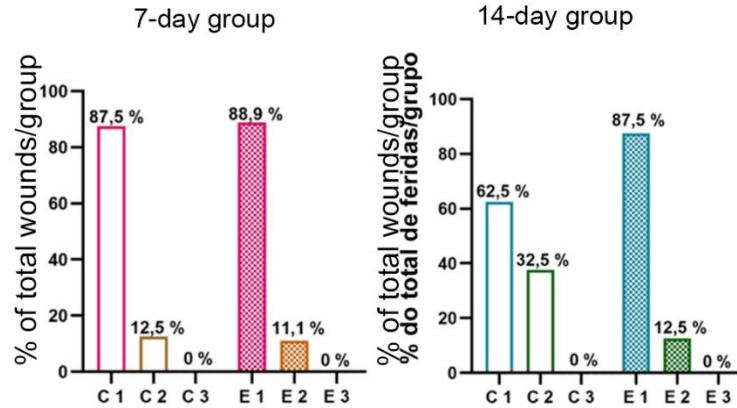


C = control group (dressing composed of sterile gauze); E = experimental group (dressing composed of nanocellulose, graphene oxide and VEGF factor). Note: Data are expressed as a percentage of the total number of wounds per group, 1 = minimum; 2 = moderate; 3 = intense

FIGURE 7 – Comparison of neutrophil exudate of subgroups seven and 14 days

Lymphoplasmacytic infiltrate

It was equal in both groups at seven days in minimum degree (C1 = 87.5% and E1 = 88.9%, Figure 8). At 14 days, it was also observed that the two groups presented identical minimum degrees (C3 = 0% and E3 = 0%, Figure 8).

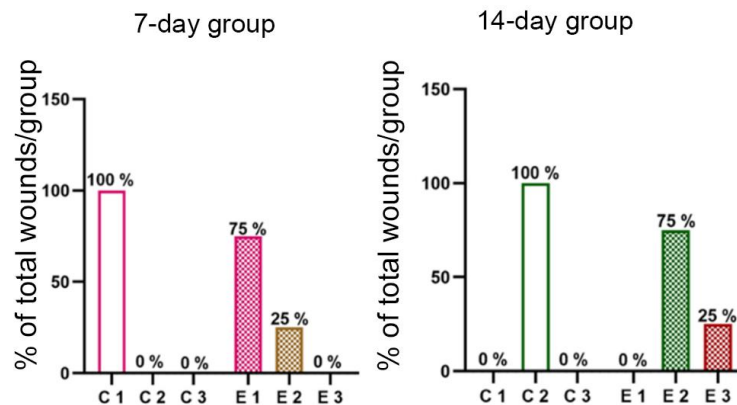


C = control group (dressing composed of sterile gauze); E = experimental group (dressing composed of nanocellulose, graphene oxide and VEGF factor). Note: Data are expressed as a percentage of the total number of wounds per group, 1 = minimum; 2 = moderate; 3 = intense.

FIGURE 2 - Comparison of lymphoplasmacytic infiltrate from subgroups seven and 14 days

Fibroblasts

Comparing the amount of fibroblasts at seven days, there was a predominance of them in a minimal degree for both group C (C1 = 100%) and group E (E1 = 75%, Figure 9). At 14 days, moderate grade predominated in both subgroups (C 2 = 100%, E 2 = 75%, Figure 9).



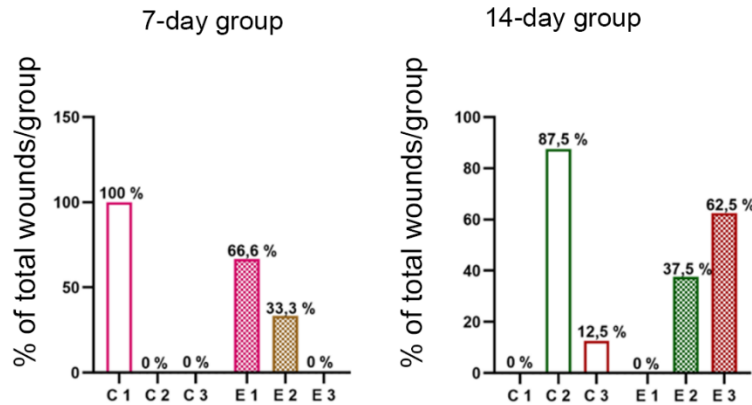
C = control group (dressing composed of sterile gauze); E = experimental group (dressing composed of nanocellulose, graphene oxide and VEGF factor). Note: Data are expressed as a percentage of the total number of wounds per group, 1 = minimum; 2 = moderate; 3 = intense.

FIGURE 9 - Comparison of fibroblasts between seven and 14 day subgroups

Collagen fibers

At seven days, 100% of the collagen fibers found in group C were classified as immature (C 1). For group E, there was a predominance of immature collagen fibers (E 1 = 86.6%), a lower percentage than in C (Figure 10). At 14 days, there was a

predominance of a mixture of immature and mature fibers (C 2 - 87.5%) in group C. In E, the predominant percentage was of mature fibers (E 3 = 62.5%) and a mixture of immature and mature fibers (E 2 = 33.3%, Figure 10).

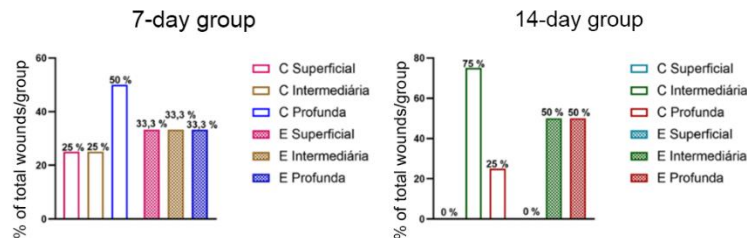


C = control group (dressing composed of sterile gauze); E = experimental group (dressing composed of nanocellulose, graphene oxide and VEGF factor). Note: Data are expressed as a percentage of the total number of wounds per group, 1 = mature fibers; 2 = mixture of immature and mature fibers; 3 = mature fibers.

FIGURE 10 – Comparison of the percentages of collagen fibers in the wounds of subgroups seven and 14 days

Depth of scars

Group C seven days had a predominance of deep scars (C 3 = 50%) in the longest and intermediate (C 2 = 25%), a number higher than group E whose results were equal between superficial, intermediate and deep (E 1, E 2, E 3 = 33.3%, Figure 11). At 14 days, there was a predominance of intermediate scar depth for group C (C 2 = 75%); for E, they were classified as equal percentages as intermediate and deep (E 2, E 3 = 50%, Figure 11).

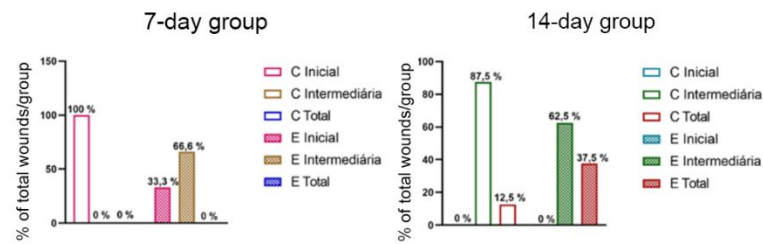


C = control group (dressing composed of sterile gauze); E = experimental group (dressing composed of nanocellulose, graphene oxide and VEGF factor). Note: Data are expressed as a percentage of the total number of wounds per group, 1 = superficial; 2 = intermediate; 3 = deep.

FIGURE 11 – Comparison of scar depth rates in groups C and E subgroups seven and 14 day

Re-epithelialization of wounds

There was a predominance of initial re-epithelialization for group C. However, in group E, 66% of it was classified as intermediate (Figure 12). At 14 days, there was a predominance of intermediate re-epithelialization for both groups. In E, there was a higher total percentage (37.5%) than in C (12.5%, Figure 12)



C = control group (dressing composed of sterile gauze); E = experimental group (dressing composed of nanocellulose, graphene oxide and VEGF factor). Note: Data are expressed as a percentage of the total number of wounds per group.

FIGURE 3 - Comparison of the rate of re-epithelialization in groups C and E subgroups seven and 14 day

DISCUSSION

In the analysis of the macroscopic aspects of the wounds in groups C and E at seven and 14 days, the dressings were properly positioned over the wounds, showed no signs of secretion, infection with or without purulent collection. The wounds were dry and crusted. Most of the time they were adhered to the wound bed. The comparison of contraction rates between groups and days showed equal contraction at seven days and a tendency to be higher at 14 days in group E, but without statistical difference. In a study conducted by De Masi et al.⁶ was found that there was a greater contraction of the wound between the 7th and 15th days similar to the present study.

In the microscopy of the first phase of healing formed by vascular proliferation, the counting performed in three fields of high magnification (400 x) of the number of capillaries allowed the quantification of the degree of neovascularization. The presence of a high number of capillaries indicates the active proliferative phase with intense angiogenesis suggestive of good initial healing evolution, with adequate oxygen and nutrient supply. The study of the group treated with nanocellulose membrane enriched with graphene oxide and growth factor showed intense angiogenesis with an increase in the total number of capillaries in relation to the control on both the 7th and 14th days, behaving efficiently considering that the proliferative phase occurs between 4-10 days.⁷

The evaluation of neutrophilic exudate is critical because it functions as a direct marker of the initial inflammatory response, a critical step in the evaluation of the efficacy and safety of any biomaterial applied to injured tissues. Neutrophils are the first cells to migrate to the site of injury, and thus studying their exudate allows us to verify whether the dressing induces excessive inflammation (indicating possible toxicity or rejection) or whether it modulates inflammation in an appropriate way, favoring healing.⁸

The present study showed that initially the percentage of neutrophilic exudate was moderate to intense and gradually decreased over the days until the end until it reached the minimum degree. The decrease at 14 days suggests the transition to the proliferative and remodeling phase, favorable to healing.

The presence of mild to moderate lymphoplasmacytic infiltrate indicates that the tissue is in the proliferative phase of healing, which usually occurs between the 4th and 7th day after the injury. Intense and persistent infiltrate, on the other hand, suggests that the tissue is being subjected to continuous antigenic stimulation, as may occur in the presence of suture material, residual necrosis, bacterial contamination, or immunological reaction to the biomaterial. Thus, the balance of this inflammatory response is key. Mild to moderate infiltrates reflect active and adequately controlled

repair, and intense infiltrates may indicate dysfunctional repair or progression to chronic inflammation. In this study, the results showed that this analysis was similar and to a minimum degree in the study group at both seven and 14 days. De Masi et al.⁶ in their study, they found the same similarity in the result, with the minimum degree of lymphoplasmacytic infiltrate.

The density of fibroblasts in the wound bed is a fundamental parameter for the evaluation of the proliferative phase. Fibroblast scarcity indicates impairment in extracellular matrix deposition and may result in delayed epithelialization. In contrast, the excessive proliferation of these cells suggests a tendency to fibrosis and dense scar formation, with consequent loss of tissue elasticity. Thus, the quantification of fibroblasts is an important morphological indicator of the proliferative stage and the volume of granulation tissue formed.

In this study, it was observed that groups C and E in the seven-day subgroup had low fibroblast density, indicating delayed healing and inadequate deposition of the extracellular matrix in this initial phase. On the other hand, at 14 days, both groups exhibited fibroblastic infiltrate to a moderate degree, reflecting adequate scar evolution and satisfactory formation of granulation tissue. Similar findings were described by De Masi et al.⁶

Collagen fibers participate in all phases of inflammatory, proliferative, and remodeling healing. Its central role is to provide a structural matrix that favors the migration of fibroblasts, keratinocytes, and endothelial cells, in addition to providing mechanical resistance to the tissue under repair. Collagen synthesis is continuous and occurs in parallel with the processes of degradation, reorganization and maturation, until the tissue reaches characteristics similar to those of the original. In general, a progressive increase in the amount of collagen fibers is expected throughout healing, but the pattern of these fibers is equally relevant. Parallel and well-organized fibers indicate mature remodeling, while disorganized fibers suggest fibrosis or pathological scarring. Thus, the analysis of the density and organization of collagen fibers allows the evaluation of the balance between synthesis and tissue remodeling.

In the present study, when comparing the amount and degree of maturation of collagen fibers at seven days, it was observed that in group C 100% of them had immature characteristics, while in group E this percentage was 86.6%. At 14 days, group C began to present a predominance of a mixture of immature and mature fibers (87.5%), indicating partial progression of remodeling. In group E, in this period, most fibers (62.5%) exhibited a mature pattern, demonstrating a more advanced collagen organization process in this phase.

There was a difference between the present study and the one described by Takejima et al.⁹ and also to the one made by Brassolatti et al.¹⁰ where there was no significant difference between the groups. However, another study¹¹ showed a higher proportion of mature collagen fibers in the group treated with cellulose film.

The depth of the scar, evaluated vertically from the base of the lesion, showed distinct behaviors between the groups over time. At seven days, group C presented predominantly deep scars, indicating slower repair and less tissue filling, while the experiment showed a balanced distribution between superficial, intermediate and deep scars, suggesting a more homogeneous scar evolution. At 14 days, group C advanced to a predominantly intermediate pattern, while group E presented equal proportions of intermediate and deep scars. Together, these findings indicate that group E demonstrated superior performance in the initial phase of healing, showing better early tissue filling, although both groups showed progression of repair over the period evaluated.

The findings suggest that, at seven days, group C presented earlier, predominantly initial re-epithelialization, while group E already showed greater progress, reaching mainly the intermediate stage of re-epithelialization. This indicates that the experimental dressing may have accelerated the transition from the initial to the intermediate phase of epithelial repair. These data corroborate those described by De Masi et al.⁶ and, also, by Takejima et al.⁹

Limitations and perspectives

The development of new materials based on nanostructured structures and high-performance composites aims to improve the mechanical strength of membranes, allow the adequate exchange of exudate and oxygen and, simultaneously, incorporate antimicrobial properties, fundamentals that motivated the present research. In this context, the elaboration of films with controlled porosity aims to facilitate fluid drainage and promote adequate aeration of the wound bed. During the study, however, excessive adherence of the films to the injured bed was observed, which made it difficult to remove them. This finding suggests the need to reevaluate the proportion of glycerol used in the formulation, in order to optimize its flexibility and reduce adhesiveness without compromising the functional performance of the material.

CONCLUSION

The findings of this study demonstrate that the nanocellulose membrane enriched with graphene oxide and vascular endothelial growth factor (VEGF) exerts a beneficial and accelerating effect on the healing process of burns in rat skin. The application of this biomaterial favored the orderly progression of the healing phases, promoting a more suitable environment for tissue repair, with evidence of efficient inflammatory modulation, stimulation of angiogenesis and structural support for the remodeling of the extracellular matrix. In general, the membrane showed superior performance than the control, suggesting translational potential as an advanced dressing. However, there is a need for additional studies to deepen the understanding of its mechanisms of action, safety, and clinical applicability.

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