Trop J Nat Prod Res, December 2025; 9(12): 6081 - 6085 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

The Effect of Dried Powdered Amnion on the Expression of M1 and M2 Macrophages and Changes in Acute Experimental Wound Area in Rats

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ARTICLE INFO

Article history:
Received 01 October 2025
Revised 19 November 2025
Accepted 29 November 2025
Published online 01 January 2026

ABSTRACT

Macrophages play a pivotal role in wound healing by coordinating inflammatory, proliferative, and tissue-remodeling phases through dynamic phenotypic shifts. This study aimed to determine the effect of dried powdered amnion (DPA) on the expression of M1 (iNOS) and M2 (CD206) macrophages, as well as changes in acute wound area in rats. A total of 63 male Wistar rats were divided into three groups: control, dried amnion sheet (DAS), and DPA, with observations conducted at 6 hours, day 2, and day 5. Wound tissues were analyzed using immunohistochemistry, while wound areas were measured using the IMITO® application. DPA produced significantly higher iNOS expression compared to other groups and demonstrated increased CD206 expression on day 2. A strong positive correlation was observed between both macrophage markers and changes in wound area, with CD206 showing a stronger relationship. These results indicate that DPA effectively modulates macrophage polarization and enhances wound healing in acute wounds.

Keywords: Amnion Powder, Inos, Cd206, Macrophage Polarization, Wound Healing

Introduction

Macrophages typically act as phagocytes to remove pathogens and cellular debris during the early phase of development, while in the advanced stage, these cells secrete mediators, such as growth factors, to support tissue regeneration.[1,2] Several studies have shown that macrophages dysfunction, such as persistent inflammation or failure of functional transition, is often a major cause of impeded healing, specifically in chronic wounds.[3] Consequently, the regulation of the cells is a strategic focus in the development of effective wound healing therapies. Polarization of macrophages into M1 (pro-inflammatory) and M2 (anti-inflammatory) phenotypes determines their function in wound healing dynamics. M1 macrophages, which are dominant in the inflammatory stage, produce cytokines, such as tumor necrolysing factor (TNF-α) and nitric oxide (NO), to fight infection and initiate the repair process.[4] Meanwhile, M2 type, which are active during proliferation and remodeling, facilitate inflammatory resolution and epithelialization through secretion of Transforming growth factor-beta (TGF- β) and Vascular Endothelial Growth Factor. These factors have been reported to support angiogenesis as well as extracellular matrix synthesis.[5,6] A coordinated transition from M1 to M2 is essential for optimal healing, and disruptions to this process often lead to chronic inflammation and impediments to tissue regeneration.[7,8]

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Citation: Ferdinand Ferdinand, Afriwardi Afriwardi, David Sontani Perdanakusuma, Hirowati Ali. The Effect of Dried Powdered Amnion on the Expression of M1 and M2 Macrophages and Changes in Acute Experimental Wound Area in Rats. Trop J Nat Prod Res. 2025; 9(12): 6081 – 6085 https://doi.org/10.26538/tjnpr/v9i12.23

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Therefore, modulation of macrophages polarization is a potential target to improve healing outcomes. Considering the central role of these cells, amniotic membrane has been recognized as a superior therapeutic biomaterial in wound care due to its immunomodulatory and proregenerative properties. Dried amnion sheet and dried powdered amnion are often obtained from the baby's amnion. Several studies have shown that amnion can direct macrophages polarization to M2 phenotype, reduce excess inflammation, and accelerate tissue regeneration through the release of cytokines, such as IL-10 and TGF-β.[8,9] With high biocompatibility and low immunogenicity, it has also proven effective in chronic wounds, including diabetic ulcers, making the membrane an ideal candidate for biologically-based therapies.[10,11] This ability shows the potential of amnion in supporting healing through direct interaction with local immune dynamics. Although dried amnion sheet is effective, its limitations in irregularly shaped wounds prompted the development of a powder as a more adaptive alternative. Clinical evidence confirms that the powder enhances the release of bioactive factors, thereby accelerating wound healing resistant to conventional therapies. Hawkins reported that the use of micronized amnion powder in diabetic foot ulcers led to improved healing with minimal contraction and superior scar aesthetics.[12] Murphy et al also showed that it outperformed hydrogel preparations and fresh sheets amnion in terms of epithelialization and tissue remodeling in a porcine model, presumably due to higher bioavailability.[13] Therefore, this study aims to investigate the effect of amnion powder on the expression of M1 and M2 macrophages, as well as impact on acute wound area changes. By analyzing biological markers of M1 (pro-inflammatory) and M2 (pro-reparative) phenotypes, the current study evaluates how amnion powder modulates macrophage polarization and supports epithelialization in acute wounds. It was hypothesized that dried amnion powder enhances M1 to M2 transition, thereby accelerating acute wound healing. The results are expected to offer insight into the molecular mechanisms underlying the efficacy of amnion powder, providing a scientific foundation for the development of more precise wound therapies, particularly in the clinical context of acute wound that requires rapid and optimal healing.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Materials and Methods

The materials used in this study include phosphate buffered formalin 4%, hematoxylin eosin, Poly-L-lysine, Xylene, Citrate buffer (pH 6), 3% H_2O_2, primary antibody; anti hiNOS dilution 1:200. anti CD206 (mannose receptor) dilution 1:200, secondary antibody, Goat Anti-Rabbit IgG Antibody, biotinylated dilution 1:100, Tris HCl buffer pH 7.6, phosphate buffer saline pH 7.4, 2% Normal Goat serum (NGS).

This study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Andalas, Padang, West Sumatra (No: 1074/UN.16.2/KEP-FK/2022). A total of 63 male Wistar rats (*Rattus norvegicus*), aged 3 months and weighing an average of 250-300 g, were used in the study, evenly divided into 3 treatment groups (Control, dried amnion sheet, dried powdered amnion) for observation at 3 different times. The dependent variables included M1 and M2 macrophages, quantified by immunohistochemistry (IHK) using iNOS (*inducible nitric oxide synthase*) and CD206 antigens (number per field of view, ratio scale). Furthermore, wound area (*raw surface*) was measured by IMITO® application[14] based on clinical photographs to determine the area in cm²-differentiated by shiny red (no epithelialization) or unshiny pink (epithelialized) surface. As independent variables, paraffin-embedded tulle (Lomatuell®) was used as a control, dried amnion sheet from the Tissue Bank of Dr. Soetomo Hospital, Surabaya, and dried powdered amnion processed at the Faculty of Pharmacy, Andalas University, Padang.

Wound tissues were harvested using chloroform at 6 hours, day 2, and day 5 post-treatment based on the consideration that external macrophages entered the wound on day 2 and the proliferation process was initiated on day 5.[15,16] The tissue extracts were then fixed with 10% formalin for anatomical pathology analysis, stained with IHK (iNOS for M1, CD206 for M2) for macrophage quantification, while wound area was measured using IMITO® before and after treatment.

Statistical Analysis

Data were processed with SPSS version 25, tested for normality through Shapiro-Wilk, then analyzed using 2-way ANOVA (Analysis of Variance) with post hoc test for normal data or Kruskal-Wallis for abnormal data, and presented in tables and graphs (SD) to support interpretation of the results.

Results and Discussion

This study evaluated the effect of Dried Powdered Amnion (DPA) on the expression of M1 macrophages (characterized by iNOS), M2 macrophages (characterized by CD206), and changes in wound area using appropriate statistical methods and the following was a summary of the findings.

iNOS expression data, which showed a normal distribution, were analyzed by 2-way analysis of variance (2-way ANOVA). The results indicated significant differences between treatment groups ($p < 0.05$) and between measurement times ($p < 0.05$). In addition, the interaction between treatment and time was also significant ($p = 0.001$), while DPA showed higher iNOS expression than other groups. Between-group comparisons showed significant differences between DPA and control ($F = 2.315$, $p = 0.041$) as well as between DPA and DSA ($F = 1.987$, $p = 0.038$). The mean iNOS expression in the DPA group was 16.20, exceeding controls (12.83) and DSA (13.73). Temporarily, iNOS expression in DPA increased from 15.62 on day 2 to 16.78 on day 5, consistently higher than controls (12.45 and 13.20) and DSA (13.10 and 14.35) (Figure 1). There was no significant interaction between DPA and DSA ($F = 0.654$, $p = 0.523$) or between DPA and control ($F = 0.789$, $p = 0.467$) in the measurement time.

CD206 expression data, which were not normally distributed, were analyzed using Kruskal-Wallis and Mann-Whitney non-parametric tests. On day 2, there was a significant difference between groups ($H = 10.017$, $p = 0.007$), with DPA recording the highest mean rating (17.00) compared to control (7.29) and DSA (8.71). A follow-up Mann-Whitney test with Bonferroni correction (threshold $p = 0.0166$) confirmed significant differences between these data sets ($U = 3.000$, $p = 0.006$) as well as between DPA and DSA ($U = 4.000$, $p = 0.009$). On day 5, the Kruskal-Wallis test showed a non-significant difference between groups ($H = 5.843$, $p = 0.054$), although the mean rating of DPA (9.93) remained higher than controls (7.64) (Figure 2) but lower than DSA (15.43). Pairwise comparisons on day 5 between DPA and control ($U = 18.000$, $p = 0.456$) and between DPA and DSA ($U = 8.000$, $p = 0.041$) were not significant. The change in CD206 expression in DPA group from day 2 (17.00) to day 5 (9.93) was also not statistically significant (Wilcoxon $Z = -1.820$, $p = 0.069$). The interaction between these data ($H = 2.134$, $p = 0.144$) as well as between DPA and control ($H = 1.876$, $p = 0.171$) over time showed no significant differences.

Table 1: Effect of Between-Subject Dependent Variable iNOS

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Square
Corrected Model	35566.343*	8	4445.793	48.181	.000	.877
Intercept	143314.651	1	143314.651	1553.152	.000	.966
Treatment	4700.347	2	2350.173	25.470	.000	.485
Time	28846.160	2	14423.080	156.308	.000	.853
Trat*Time	2019.836	4	504.959	5.472	.001	.288
Error	4982.836	54	92.273			
Total	183863.760	63				
Corrected Total	40549.109	62				

a. R Squared = .877 (Adjusted R Squared = .859

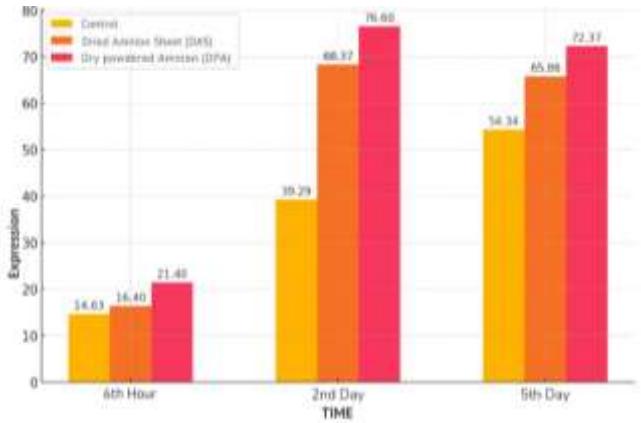

Figure 1: Bar graph depicting the expression of iNOS (M1 macrophage marker) in three treatment groups (control, DAS, DPA) at three measurement times (6 hours, 2nd day, 5th day) in rats (n = 7 per group)

The relationship between changes in wound area and iNOS expression was examined by Spearman correlation, showing a significant positive association ($r = 0.501$, $p < 0.001$) (Figure 3, 4). Simple linear regression analysis yielded a coefficient (B) of 0.474, $R^2 = 0.278$, and $p < 0.001$, with the equation, Wound Change = 21.825 + 0.474 × iNOS Expression. For DPA group with a mean iNOS of 16.20, the predicted wound area change was 29.50. Furthermore, the relationship between wound area changes and CD206 expression showed a significant positive correlation ($r = 0.579$, $p < 0.001$), with regression analysis yielding $B = 0.548$, $R^2 = 0.392$, and $p < 0.001$. The regression equation was Wound Change = 16.371 + 0.548 × CD206 Expression. For DPA, the predicted wound area changes at day 2 (mean rank = 17.00) were 25.69, and at day 5 (mean rank = 9.93) were 21.81.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 2: Analysis Tukey HSD[a,b] between groups

Treatment	N	1	2
Control	21	36.0857	
DAS	21		50.2095
DPA	21		56.7905
Sig.		1.000	.077

The error term is Mean Square (Error) = 92.273

In comparing the effect on wound area change in Table 1, 2 and 3, CD206 showed a stronger effect (R = 0.626, R^2 = 0.392, B = 0.548) than iNOS (R = 0.528, R^2 = 0.278, B = 0.474). DPA group consistently showed higher iNOS expression than controls and DSA, as well as significantly increased CD206 expression at day 2, although at day 5 it was lower.

DPA correlated with higher iNOS expression compared to control and DAS on day 2 and day 5, and significantly increased CD206 expression on day 2. There was no significant interaction between DPA and time or other groups for either marker. CD206 showed a greater influence on changes in wound area (Table 4, 5), with a prominent contribution of DPA, specifically on day 2. DPA showed great potential supporting the wound healing process through its ability to modulate macrophage activity. These were key cells in tissue regeneration, specifically in wound healing, and had 2 main phenotypes, namely M1, which was pro-inflammatory, and M2, which was anti-inflammatory. Each type of macrophage played a role in different stages of wound healing. This study proved that DPA increased the expression of iNOS (Figure 5) as a marker of M1 macrophages to support wound clearance and simultaneously promoted the expression of CD206 (Figure 6) as a marker of M2 macrophages for wound healing.

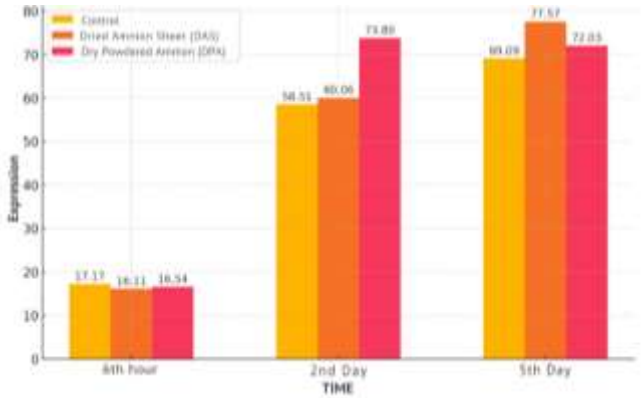

Figure 2: Bar graph showing the expression of CD206 (M2 macrophage marker) in three groups (control, DAS, DPA) at three times (6 hours, 2nd day, 5th day) in Rats (n = 7 per group)

In the early stages of wound healing, M1 macrophages played an important role in the initial preparation for wound healing. DPA was shown to increase iNOS expression, which amplified the inflammatory response to disinfect the wound. This process involved the production of cytokines such as TNF-α and IL-12, which not only cleansed the wound of pathogens but also recruited other immune cells to the site of injury.[4,1] Furthermore, DPA-induced M1 macrophages could affect epithelial cells, promoting epithelial cell migration and proliferation as a crucial step in wound epithelialization shown in tissue culture studies.[16,17]

Table 3: Analysis Tukey HSD[a,b] between times

Treatment	N	1	2
6th hour	21	17.4762	
2nd day	21		61.4190
5th day	21		64.1905
Sig.		1.000	.621

The error term is Mean Square (Error) = 92.273

Excessive and prolonged M1 activity could be a double-edged sword. Sustained inflammation due to high iNOS expression could potentially inhibit granulation tissue formation and collagen deposition, therefore slowing healing.[4] Although DPA effectively supported M1 role in the early phase, transitioning to M2 phenotype was an important step to ensure optimal healing.

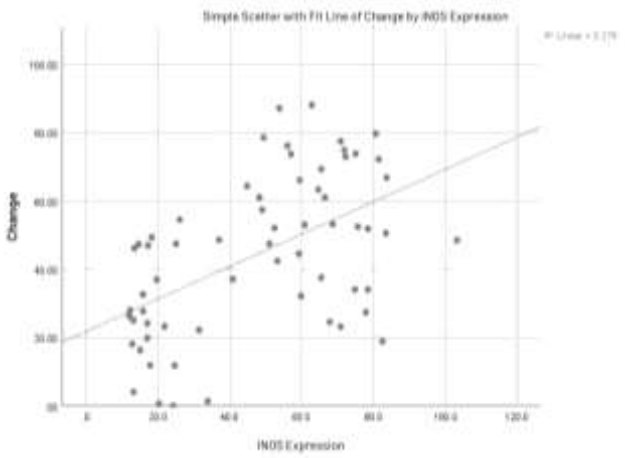

Figure 3: Scatterplot with regression line depicting the relationship between iNOS expression and change in wound area (cm²) in Rats. The data points show a positive correlation (r = 0.501, p < 0.001), with a linear regression line (R^2 = 0.278) indicating that increased iNOS expression correlates with increased wound area change

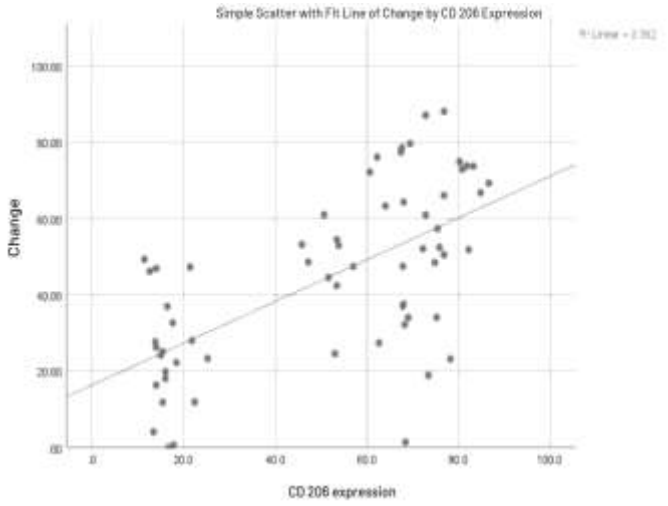

Figure 4: Scatterplot with regression line showing the relationship between CD206 expression and change in wound area (mm²) in Rats. The data points indicate a positive correlation (r = 0.579, p < 0.001), with a linear regression line (R^2 = 0.392) showing that increased CD206 expression correlates with increased change in wound area

In addition to supporting M1 macrophages, DPA also facilitated the switch to M2 macrophages, characterized by a significant increase in CD206 expression on the second day post-treatment. This was crucial in relieving inflammation and initiating tissue repair. M2 anti-inflammatory macrophages supported healing through 3 main mechanisms, namely antifibrotic effect, immune modulation, and tissue remodeling. In terms of antifibrotic, M2 macrophages inhibited fibrosis through a paracrine mechanism involving IL-6, suppressed the expression of genes such as collagen types 1 and 3, and *connective*

tissue growth factor, and also increased *matrix metalloproteinase 1* activity to remodel the extracellular matrix[18].

From the immune side, M2 macrophages strengthened immune tolerance and reduced excess inflammation.[19] M2 macrophages carried out this remodeling process by rearranging collagen fragments to reshape post-traumatic tissue.[20] Amnion preparations inhibited pro-inflammatory pathways such as Toll-like receptors and Tumor necrosis factor[21] while activating PI3K/AKT/HIF-1α anti-inflammatory pathway.[22] Human amnion mesenchymal cells (hAMTCs) also accelerated the M1 to M2 transition by increasing M2 markers and suppressing pro-inflammatory cytokines.[23] The enhancement of CD206 by DPA confirmed its role as a potential agent in wound healing.

Table 4: Analysis CD206 in Wounds (Ranks)

	Group	N	Mean Rank
Value	Control		7.29
	DAS	7	8.71
	DPA	7	17.00
	Total	21	

The expression of iNOS and CD206 showed a strong correlation with the rate of extensive epithelialization to close the wound, with CD206 having a more significant relationship. iNOS, as M1 marker, supported wound clearance in the early inflammatory phase, but when not controlled, it could worsen the healing outcome due to prolonged inflammation.[4] However, CD206, as an M2 marker, played a dominant role in inflammation resolution, tissue remodeling, and fibrosis prevention, all of which supported effective healing.[18,24]

DPA appeared to optimize this dynamic by increasing iNOS to disinfect wound at an early stage, followed by an increase in CD206 to accelerate tissue repair. A therapeutic approach that accelerated M1 to M2 transition was shown in studies using amniotic hydrogel preparations in chronic wounds such as diabetic ulcers.[20] External factors, such as extracellular matrix stiffness, also influenced this process. In soft matrices, M1 macrophages promoted epithelial proliferation, while this effect was reduced in rigid matrices [25,26].

Table 5: Test Statistics[a,b] (Value) of CD206

Kruskal-Wallis H	10.017
Df	2
Asymptotic Significance	.007

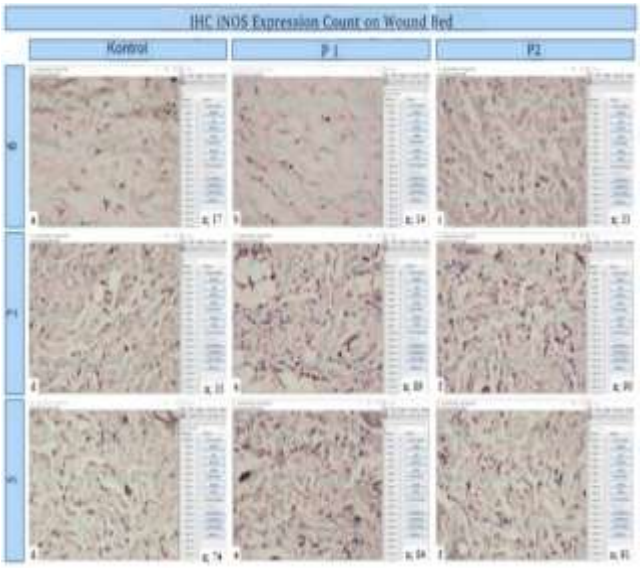

Figure 5: Histopathological images of iNOS expression (M1 macrophage marker) in wound granulation tissue for three groups at three times (6 hours, 2 days, 5 days) in rats. Black color indicates iNOS expression, with cell counts (n=17 to n=91). Expression appears to increase over time, especially in P2, suggesting a role in the wound inflammatory process

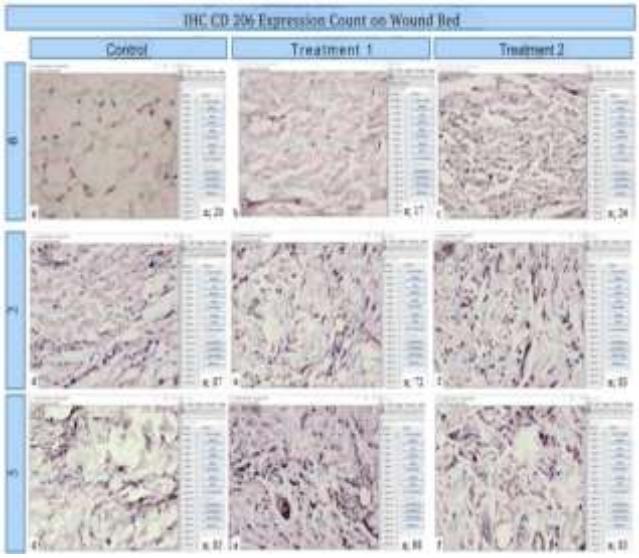

Figure 6: Histopathological image of CD206 expression in wound granulation tissue in three groups (control, DPA, DAS) at three times (6 hours, 2 days, 5 days) in Rats. Black color indicates the expression of CD206 (M2 macrophage marker), with cell counts (n=20 to n=85). Expression increased over time, especially in P2, supporting a role in wound healing

Therefore, the interaction between iNOS and CD206, which was modulated by DPA, reflected the complexity of wound healing that needed to be continuously explored. This study had several weaknesses that required consideration. First, although DPA demonstrated effectiveness in modulating M1 and M2 macrophage expression and accelerating acute wound healing in rats, the relatively short duration of observation (5 days) limited understanding of long-term effects, such as potential fibrosis or scar tissue stability. Secondly, this study focused only on iNOS and CD206 markers, while specific cytokines such as TNF-α, IL-10, or TGF-β that played a role in macrophage polarization were not directly measured, and the molecular mechanisms underlying the effects of DPA had not been fully revealed. Thirdly, this study was conducted in rat model with acute wounds, which could not fully reflect chronic wound conditions in humans, such as diabetic ulcers, which had higher pathophysiological complexity

Conclusion

In conclusion, DPA effectively promoted acute wound healing in rats by modulating macrophage polarization. DPA significantly increased iNOS expression (M1 macrophage marker) to support wound clearance in the early inflammatory phase and CD206 expression (M2 macrophage marker) to accelerate inflammatory resolution and tissue regeneration, specifically on day 2 post-treatment. The strong positive correlation between these 2 markers and changes in wound area suggested that DPA optimized healing dynamics, with CD206 having a greater influence than iNOS. These results confirmed the potential of DPA as an innovative biological therapy for acute wound management.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. DiPietro LA. Wound healing: the role of the macrophage and other immune cells. Shock. 1995;4(4):233–240. https://doi.org/10.1097/00024382-199510000-00001
2. 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;9:419. https://doi.org/10.3389/fphys.2018.00419
3. Koh TJ, DiPietro LA. Inflammation and wound healing: the role of the macrophage. Expert Rev Mol Med. 2011;13:e23. https://doi.org/10.1017/S1462399411001943
4. Gao X, Lu C, Miao Y, Ren J, Cai X. Role of macrophage polarisation in skin wound healing. Int Wound J. 2023;20(7):2551–2562. https://doi.org/10.1111/iwj.14119
5. Al Sadoun H. Macrophage phenotypes in normal and diabetic wound healing and therapeutic interventions. Cells. 2022;11(15):2430. https://doi.org/10.3390/cells11152430
6. Hassanshahi A, Moradzad M, Ghalamkari S, Fadaei M, Cowin AJ, Hassanshahi M. Macrophage-mediated inflammation in skin wound healing. Cells. 2022;11(19):2953. https://doi.org/10.3390/cells11192953
7. Rahavian AH, Hazrati E, Azar DA, Allameh F, Hojjati SA, Javanmard B, Hamidi R. Using dried human amniotic membrane in secondary intention wound healing after urological cancer surgery: the first randomized clinical trial in Iran. Int J Cancer Manag. 2021;14(5):e111421. https://doi.org/10.5812/IJCM.111421
8. Subhan BS, Kwong J, Kuhn JF, Monas A, Sharma S, Rabbani PS. Amniotic fluid-derived multipotent stromal cells drive diabetic wound healing through modulation of macrophages. J Transl Med. 2021;19(1):32. https://doi.org/10.1186/s12967-020-02674-5
9. Ebrahimi L, Samadikuchaksaraei A, Joghataei MT, Safa M, Abtahi Froushani SM, Ghasemian M, Zolfaghari S, Mozafari M, Brouki MP. Transplantation of decellularised human amniotic membranes seeded with mesenchymal stem cell-educated macrophages into animal models. J Biomed Mater Res B Appl Biomater. 2022;110(7):1637–1650. https://doi.org/10.1002/jbm.b.35024
10. Corrêa MEAB, Silveira PCL. Amniotic membrane in wound healing: new perspectives. J Wound Care. 2024;33(8):612–616. https://doi.org/10.12968/jowc.2022.0054
11. Russo A, Bonci P, Bonci P. The effects of different preservation processes on the total protein and growth factor content in a new biological product developed from human amniotic membrane. Cell Tissue Bank. 2012;13(3):353–361. https://doi.org/10.1007/s10561-011-9261-5
12. Hawkins B. The use of micronized dehydrated human amnion/chorion membrane allograft for the treatment of diabetic foot ulcers: a case series. Wounds. 2016;28(5):152–157.
13. Murphy SV, Skardal A, Nelson RA, Sunnon K, Reid T, Clouse C, Kock ND, Jackson J, Soker S, Atala A. Amnion membrane hydrogel and amnion membrane powder accelerate wound healing in a full thickness porcine skin wound model. Stem Cells Transl Med. 2020;9(1):80–92. https://doi.org/10.1002/sctm.19-0101
14. Biagioni RB, Carvalho BV, Manzioni R, Matielo MF, Brochado NFC, Sacilotto R. Smartphone application for wound area measurement in clinical practice. J Vasc Surg Cases Innov Tech. 2021;7(2):258–261. https://doi.org/10.1016/j.jvscit.2021.02.0081
15. 15. DiPietro LA, Polverini PJ, Rahbe SM, Kovacs EJ. Modulation of JE/MCP-1 expression in dermal wound repair. Am J Pathol. 1995;146(4):868-875.
16. Engelhardt E, Toksoy A, Goebeler M, Debus S, Bröcker E-B, Gillitzer R. Chemokines IL-8, GROα, MCP-1, IP-10, and Mig are sequentially and differentially expressed during phase-specific infiltration of leukocyte subsets in human wound healing. Am J Pathol. 1998;153(6):1849-1860. doi:10.1016/S0002-9440(10)65699-4
17. Chen C, Liu T, Tang Y, Luo G, Liang G, He W. Epigenetic regulation of macrophage polarization in wound healing. Burns Trauma. 2023;11:tka057. https://doi.org/10.1093/burnst/tkac057
18. Gindele JA, Mang S, Pairet N, Christ I, Gantner F, Schymeinsky J, Lamb DJ. Opposing effects of in vitro differentiated macrophages sub-type on epithelial wound healing. PLoS One. 2017;12(9):e0184386. https://doi.org/10.1371/journal.pone.0184386
19. Zmuda H, Pathak A. Epithelial multicellular clustering enabled by polarized macrophages on soft matrices. FASEB J. 2023;37(8):e12012. https://doi.org/10.1096/fj.202300120rr
20. Kurachi I, Kurita E, Takushima A, Suga H. Human CD206+ macrophages show antifibrotic effects on human fibroblasts through an IL-6–dependent mechanism in vitro. Plast Reconstr Surg. 2020;147(2):231e–239e. https://doi.org/10.1097/prs.0000000000007563
21. Yang Y, Nagai S, Kang S, Xia Y, Kawano Y, Miyake K, Karasuyama H, Azuma M. Tolerogenic properties of CD206+ macrophages appeared in the sublingual mucosa after repeated antigen-painting. Int Immunol. 2020;32(8):509–518. https://doi.org/10.1093/intimm/dxaa014
22. Faust MT, Sadtler K, Destefano S, Fertil D, Josyula A. The role of macrophage scavenger receptors in engineered biomaterial scaffold remodeling. J Immunol. 2023;210(Suppl 1):71.45–71.45. https://doi.org/10.4049/jimmunol.210.supp.71.45
23. Zhang T, Shao M, Li H, Chen X, Zhang R, Wu J, Wang J, Guo Y. Decellularized amnion membrane triggers macrophage polarization for desired host immune response. Adv Healthc Mater. 2024;13(29):e202402139. https://doi.org/10.1002/adhm.202402139
24. Song X, Xiao J, Wu J, Sun L, Chen L. Decellularized amniotic membrane promotes the anti-inflammatory response of macrophages via PI3K/AKT/HIF-1α pathway. Chin Chem Lett. 2025;36(1):109844. https://doi.org/10.1016/j.cclet.2024.109844
25. Magatti M, Vertua E, De Munari S, Caro M, Caruso M, Silini A, Delgado M, Parolini O. Human amnion favours tissue repair by inducing the M1-to-M2 switch and enhancing M2 macrophage features. J Tissue Eng Regen Med. 2017;11(10):2895–2911. https://doi.org/10.1002/term.2193
26. Jang TH, Park SC, Yang JH, Kim JY, Seok JH, Park US, Choi CW, Lee SR, Han J. Cryopreservation and its clinical applications. Integr Med Res. 2017;6(1):12–18. https://doi.org/10.1016/j.imr.2016.12.001
27. Mohr A, Besser M, Broichhausen S, Winter M, Bungert AD, Strücker B, Juratli MA, Pascher A, Becker F. The influence of apremilast-induced macrophage polarization on intestinal wound healing. J Clin Med. 2023;12(10):3359. https://doi.org/10.3390/jcm12103359
28. Sun D, Chang Q, Lu F. Immunomodulation in diabetic wounds healing: the intersection of macrophage reprogramming and immunotherapeutic hydrogels. J Tissue Eng. 2024;15:20417314241265202. https://doi.org/10.1177/20417314241265202