Proceedings of ICBioMed 2026 Symposium: Biomedical Engineering and Smart Medicine
DOI: 10.54254/2753-8818/2026.HZ38087

Macrophage Modulation for Regenerative Skin Repair Beyond Accelerated Wound Closure: An Evidence Hierarchy Analysis of Preclinical Full-Thickness Wound Research

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Abstract. Adult full-thickness skin wounds usually heal by fibrosis rather than complete restoration. Macrophages regulate inflammation, angiogenesis, fibroblast activity, and extracellular-matrix remodeling, making them therapeutic targets. This paper asks whether local macrophage-modulating interventions in preclinical full-thickness excisional wounds can improve the regenerative quality of repair beyond wound closure. A structured evidence-hierarchy analysis independently classified macrophage mechanisms and tissue outcomes in 12 core studies while considering model, comparator, timing, and follow-up. Seven studies provided functional or causal macrophage evidence and six reported at least one positive regenerative-quality outcome. Only Hu et al. provided a positive linkage within the same comparison: human-monocyte delivery reduced gross scar area, but scar depth, collagen quality, and tensile strength did not improve consistently. Other causal studies clustered around closure, re-epithelialization, angiogenesis, and matrix formation; higher-level outcomes usually lacked macrophage-specific causal testing. Negative and discordant findings showed that faster closure or M2-associated polarization did not necessarily predict tissue restoration. Strong regeneration claims therefore require macrophage causality and regenerative-quality endpoints to converge within the same comparison, with appropriate controls and remodeling follow-up.

Keywords: macrophage modulation, skin regeneration, wound healing, evidence hierarchy, regenerative medicine

1. Introduction

Full-thickness skin injury in adult mammals usually heals through fibrotic repair and scar formation rather than complete restoration of native skin structure and function. Skin appendages, organized extracellular matrix (ECM), barrier properties, and biomechanical function may remain incompletely restored after closure. Macrophages participate throughout inflammatory, proliferative, and remodeling phases by regulating inflammation resolution, angiogenesis, fibroblast behavior, and ECM remodeling, making them important targets in skin repair and tissue engineering. However, macrophage activation should not be reduced to a fixed "M1 pro-inflammatory/M2 reparative" binary because phenotype and function depend strongly on time and microenvironment [1]. In

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DOI: 10.54254/2753-8818/2026.HZ38087

addition, murine wounds can close substantially through tissue contraction, so closure speed alone may overestimate the extent of regenerative recovery [2].

Local IL-1β blockade can shift wound macrophages toward a healing-associated phenotype and improve diabetic wound healing [3]. Yet stronger macrophage manipulation does not necessarily produce higher-quality tissue restoration. Exogenous M2 macrophages delayed repair in diabetic wounds [4], while direct macrophage delivery accelerated closure without corresponding improvements in several scar, collagen-quality, and mechanical outcomes [5]. This paper therefore evaluates macrophage evidence and tissue-regeneration outcomes as independent dimensions while accounting for study design and follow-up. It contributes a dual-dimension operational framework that specifies the minimum evidence combination for a macrophage-dependent regeneration claim, guiding the design and interpretation of preclinical skin-repair studies.

2. Analytical framework for macrophage-mediated skin repair evaluation

2.1. Evidence scope and study selection criteria

This analysis focuses on local wound-directed interventions in mammalian full-thickness excisional cutaneous wounds. The 12-study core set was selected for direct relevance and nonredundant mechanistic, regenerative-outcome, negative, or discordant evidence; interpretable macrophage-related findings and tissue-level outcomes were required. Non-diabetic and diabetic or impaired-healing models are treated as distinct contexts, while infected wounds are contextual only because antimicrobial effects may independently alter inflammation and closure. The analysis compares macrophage evidence strength with tissue-outcome quality rather than ranking interventions.

2.2. Hierarchy of macrophage mechanistic evidence

For this analysis, macrophage evidence is classified using a study-specific operational scale. M-I represents descriptive associations, including single-marker, single-time-point, or poorly identity-resolved signals. M-II represents identity-resolved, multidimensional, or time-resolved evidence, as demonstrated by co-localization, flow cytometry, multiple phenotype markers, single-cell analysis, or repeated temporal assessment. M-III denotes functional or causal evidence, including depletion, adoptive transfer, macrophage-specific genetic manipulation, or macrophage-targeted blocking or rescue linked directly to wound outcomes. This M-I-M-III scale is neither a field-wide standard nor a fixed M1/M2 classification [1]. Cross-tabulation with the independent R1-R3 scale prevents mechanistic strength from predetermining outcome quality.

2.3. Hierarchy of repair and regenerative outcomes

For this analysis, tissue outcomes are independently classified using a second study-specific operational scale. R1 includes wound area, closure rate, and time to closure. R2 includes conventional repair outcomes such as re-epithelialization, granulation tissue, angiogenesis, and general collagen deposition. R3 denotes regenerative-quality restoration, including native-like ECM or collagen architecture, quantified scar or fibrosis reduction, wound-bed appendage neogenesis, barrier or biomechanical recovery, and longer-term structural or functional normalization. R1-R3 is a study-specific, not established clinical, classification. Cross-tabulation with M-I-M-III tests same-comparison convergence of macrophage causality and regenerative-quality restoration; murine closure also requires attention to contraction and splinting [2].

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3. Evidence profile of macrophage modulation in wound repair

Table 1 summarizes the mechanistic strength and the highest tissue outcome directly linked to it within each relevant experimental comparison. Across the 12-study core set, seven studies provided functional or causal macrophage evidence and six reported at least one positive regenerative-quality outcome. Only one study contained a positive same-comparison M-III-to-R3 signal, and that signal was limited and internally mixed [5]. These counts describe the selected analytical core, not field-wide prevalence.

3.1. Overall distribution across the dual-dimension framework

Conventional repair dominated: inflammatory blockade, cell delivery, fat grafting, biomaterials, and cytokine treatment improved closure, re-epithelialization, angiogenesis, or matrix formation [3, 5-8]. Jetten et al. provided a counterexample: M2a or M2c transfer failed to improve wild-type wounds and delayed diabetic healing [4]. Thus, neither stronger macrophage intervention nor an M2 label is a monotonic predictor of benefit.

Table 1. Comparison-level macrophage evidence and tissue-repair outcomes

Study	Model context	Macrophage evidence	Linked tissue outcome	DirectM-III-to-R3linkage	Key design qualifier
Mirza et al. [3]	Diabetic mouse	M-II	R2+	No	IL-1β blockade, macrophage-associated repair
Jetten et al. [4]	Wild-type + db/db mouse	M-III	R2- (negative)	No	Negative result: M2a/M2c transfer failed to improve wild-type healing and delayed diabetic healing
Hu et al. [5]	Wild-type, diabetic + immunodeficient mice	M-III	R2+,R3 mixed	Yes, limited	Splinted, monocyte arms reduced gross scar area, but other R3 measures were null
Wang et al. [6]	Diabetic rat	M-III	R2+	No	Macrophage depletion, collagen alignment qualitative
Zhu et al. [7]	Full-thickness mouse	M-III	R2+	No	Macrophage depletion, linked endpoints remain R2
Tao et al. [8]	Diabetic mouse	M-III	R2+	No	Macrophage-specific IL-27Rα knockout
Zuo et al. [9]	Full-thickness mouse	M-III	R1/R2+	No	Depletion-supported mechanism, linked endpoints R1/R2
He et al. [10]	Non-diabetic mouse	M-II	R3+	No	R3 positive, no macrophage-specific perturbation
Zheng et al. [11]	Non-diabetic rat	M-II	R3+	No	MSC-free hydrogel comparator, macrophage association
Tan et al. [12]	Diabetic mouse	M-II	R3+	No	Barrier endpoint, no macrophage-specific perturbation

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Table 1. (continued)

Li et al. [13]	Full-thickness + scar models	M-II	R3+	No	Splinted, scar endpoint, no macrophage-specific perturbation
Savitri et al. [14]	Non-diabetic mouse	M-II main arm, separate M-III	R3+ main arm,R2dependence	Not established	R3 main arm; strongest R3 endpoint not tested in depletion comparison

Note. M-I = descriptive macrophage association; M-II = identity-resolved, multidimensional, or time-resolved evidence; M-III = functional or causal macrophage evidence. R1 = wound closure; R2 = conventional tissue repair; R3 = regenerative-quality structural or functional restoration. Direct M-III-to-R3 linkage required a macrophage-lineage causal perturbation to alter a positive R3 endpoint within the same comparison. Hu et al. met this criterion only for reduced gross scar area in human-monocyte arms; other R3 measures were null. Qualitative collagen alignment without native-skin benchmarking was not classified as R3. The 12 studies form a selected analytical core rather than an exhaustive field sample.

3.2. Mismatch pattern 1 strong macrophage causality usually without R3 confirmation

A recurrent pattern is the concentration of M-III studies at R1-R2 rather than R3. Macrophage depletion, cell transfer, metabolic perturbation, and macrophage-specific receptor manipulation established functional or causal involvement, yet most linked outcomes remained closure, vascularization, re-epithelialization, or matrix formation [4-9]. Hu et al. provided a limited exception: human-monocyte delivery reduced gross scar area, but other R3 measures were unchanged [5].

Mechanistic confidence and regenerative endpoint quality are independent dimensions. M-III evidence can establish macrophage involvement in healing without demonstrating restoration of native-like structure or function. Therefore, M-III should support a claim of macrophage-dependent repair unless an R3 endpoint is directly altered by the same causal perturbation.

3.3. Mismatch pattern 2 R3 outcomes without direct macrophage causality

The opposite mismatch occurs in studies reporting hair-follicle formation, improved tissue architecture, scar reduction, barrier recovery, or other R3 outcomes [10-14]. These findings provide stronger evidence of regenerative-quality repair than closure alone, but macrophage involvement is usually demonstrated through phenotype shifts, identity-resolved analysis, or separate mechanistic experiments rather than through a causal perturbation directly tied to the R3 endpoint.

Savitri et al. exemplify this boundary: depletion supported macrophage dependence for R2 remodeling, but the strongest R3 endpoint was not tested within the same depletion comparison [14].

Within this core set, the mismatch largely reflects study architecture. Causal experiments tended to assess early or conventional repair, whereas R3-focused biomaterial studies assessed later structural outcomes but usually treated macrophage states as associated mechanisms [4-14]. Short follow-up, contraction-sensitive models, non-equivalent comparisons, and limited functional assessment restricted same-chain inference. Hu et al. provide limited convergence, but their mixed R3 results do not address the broader gap [5]. Figure 1 summarizes these comparison-level evidence patterns.

DOI: 10.54254/2753-8818/2026.HZ38087

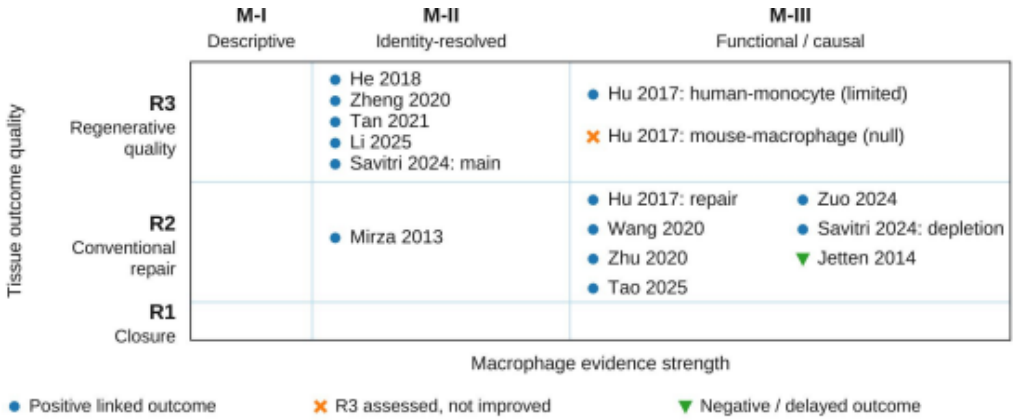

Figure 1. Macrophage evidence strength and tissue-outcome quality

Note. Markers represent the highest tissue outcome linked to macrophage evidence within each comparison. Hu 2017 is split by experimental arm: human-monocyte delivery reduced gross scar area, whereas mouse-macrophage arms did not improve assessed R3 measures. Jetten 2014 denotes a negative or delayed outcome. Savitri 2024 is split between the main intervention and separate depletion comparisons. M-I-M-III and R1-R3 are discrete categories; within-cell positions only separate labels.

4. Standards for justifying regenerative repair claims

4.1. Faster closure is not equivalent to better regeneration

Hu et al. illustrate why wound closure and healed-tissue quality should be considered separately [5]. In splinted wounds, macrophage or monocyte delivery accelerated closure. Mouse-macrophage arms did not improve scar dimensions, tensile strength, or collagen quality. Human-monocyte arms reduced gross scar area, creating the core set's only positive same-comparison M-III-to-R3 signal, but scar depth, collagen quality, and tensile strength remained unchanged. Thus, even with contraction control and an unseeded scaffold comparator, one favorable scar metric did not establish broad restoration of native architecture or function.

4.2. More "M2" is not necessarily more regenerative

The evidence also challenges a simple interpretation in which stronger M2-associated polarization necessarily produces better healing. Direct administration of ex-vivo generated M2a or M2c macrophages failed to improve healing in wild-type wounds and delayed repair in diabetic wounds [4]. This finding shows that a macrophage phenotype defined before transplantation cannot be assumed to retain the same function within a changing wound environment. Macrophage function also depends on broader biological programs. Zuo et al. linked reparative activity to PPARγ-associated lipid metabolism, indicating that function cannot be inferred from a few phenotype markers alone [9]. Conversely, the LA-peptide study demonstrated that restraining excessive TGF-β-associated macrophage-fibroblast signaling reduced scar-related outcomes [13]. Together, these findings emphasize timing, functional state, wound context, and stromal responses over a simple unidirectional M1-to-M2 shift.

4.3. Minimum evidence combination for valid regeneration conclusions

Neither macrophage causality nor an R3 outcome is sufficient alone. A defensible macrophage-dependent regeneration claim requires three conditions:

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(1) Macrophage causality. A functional or causal perturbation must support macrophage involvement whenever causal language is used.

(2) Regenerative endpoint. The experiment must prespecify at least one R3 outcome rather than infer regeneration from closure, angiogenesis, or collagen amount.

(3) Same-comparison linkage. The macrophage perturbation itself must alter that R3 endpoint within the same experimental comparison.

These conditions prevent two inference errors: treating M-III evidence limited to R1-R2 as proof of regeneration, and treating an R3-positive outcome as macrophage dependent when macrophages were measured only associatively. Savitri et al. illustrate the latter boundary because the strongest R3 endpoint was not tested within the depletion comparison [14]. Appropriate intervention-specific controls, contraction-aware models, and remodeling-phase follow-up can further strengthen the inference. This criterion complements broader systematic catalogues of immunomodulatory tissue-engineering strategies [15] by testing the evidentiary validity of regeneration claims.

5. Conclusion

Within the core preclinical evidence evaluated here, macrophage modulation clearly influences cutaneous wound healing, but the evidence is stronger for accelerated repair than for the restoration of native-like skin. Seven of the 12 core studies provided functional or causal macrophage evidence and six reported at least one positive regenerative-quality outcome. Only Hu et al. contained a positive same-comparison link, limited to reduced gross scar area in human-monocyte-treated immunocompromised mice; scar depth, collagen quality, and tensile strength did not improve consistently. Most causal studies remained centered on closure, re-epithelialization, angiogenesis, or matrix formation, whereas higher-level outcomes often lacked direct macrophage-specific causal testing.

The evidence also argues against a simple model in which stronger M2-associated polarization necessarily produces better regenerative outcomes. Direct administration of M2-polarized macrophages did not consistently improve healing, while other studies linked macrophage function to metabolic state, timing, wound context, and stromal interactions. Excessive repair-associated signaling may also contribute to fibrosis. Macrophage modulation should therefore be interpreted as a dynamic process rather than as a unidirectional M1-to-M2 transition.

A convincing claim of macrophage-dependent regenerative repair should combine causal macrophage evidence with a prespecified regenerative-quality endpoint within the same experimental comparison. Appropriate controls, contraction-aware wound models, and sufficient remodeling follow-up further strengthen this inference. The present analysis remains limited by heterogeneity among animal models, interventions, and follow-up periods and was not designed as an exhaustive PRISMA-compliant systematic review. Future studies should directly test whether temporally controlled macrophage perturbation alters defined structural and functional regeneration endpoints, clarifying when macrophage modulation merely accelerates repair and when it genuinely supports restoration of native-like skin.

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