WHIOCE PUBLISHING PTE. LTD.
PROVIDING
FIRST-CLASS SCIENTIFIC INFORMATION
FOR TOP SCHOLARS

2026 Volume 11, Issue 4
Advances in Precision Medicine
ISSN: 2424-9106 (Online)
ISSN: 2424-8592 (Print)

Sample-level Single-cell Transcriptomic Analysis Identifies Molecular Features Associated with Non-healing Diabetic Foot Ulcers

Jiaqi Lian[1], Chunyan Ren[2]*, Xinyu Zou[1]*

[1]Orthopedic Surgery Department, The First Affiliated Hospital of Dalian Medical University, Dalian 116000, Liaoning, China

[2]Department of Wound Repair, Zhangqian Road Branch, The First Affiliated Hospital of Dalian Medical University, Dalian 116000, Liaoning, China

Authors to whom correspondence should be addressed.

Copyright: © 2026 Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), permitting distribution and reproduction in any medium, provided the original work is cited.

Abstract: *Background:* Diabetic foot ulcers (DFUs) frequently fail to heal, but the local cellular programs associated with non-healing remain incompletely resolved. We performed a conservative secondary analysis of public single-cell RNA-seq count matrices to identify sample-level signatures and candidate transcripts associated with non-healing DFU. *Methods:* Raw count matrices from GSE165816 were summarized at the sample level. The primary analysis focused on 33 foot-skin specimens, including 9 healing DFU, 5 non-healing DFU, 8 diabetic non-DFU, and 11 non-diabetic foot-skin samples. Marker-based signatures representing fibroblast activation, extracellular matrix remodeling, angiogenesis, keratinocyte activation, and immune states were calculated from log2 counts per million. Healing and non-healing DFU samples were compared using Welch statistics at the pseudobulk level. *Results:* The analyzed foot-skin matrices contained 94,325 cells. Non-healing DFU samples showed broad remodeling of immune- and matrix-associated pseudobulk expression. A compact data-driven marker panel (IGHG3, IGLC2, IGKC, IGHG1, MMP3, IGHA1) separated healing from non-healing DFU samples with an apparent sample-level AUC of 0.89, although this estimate should be interpreted cautiously because of the modest number of independent DFU specimens. *Conclusion:* Public single-cell DFU data support the presence of distinct sample-level molecular states in non-healing ulcers. The analysis prioritizes transcripts and signatures for low-cost follow-up studies while emphasizing the need for patient-level validation.

Keywords: Diabetic foot ulcer; Single-cell RNA sequencing; Non-healing wound; Pseudobulk; Biomarker; Extracellular matrix

Online publication: April 26, 2026

1. Introduction

Diabetic foot ulcers are a major complication of diabetes and are clinically important because failure to heal increases the risk of infection, hospitalization, and lower-extremity amputation. The wound bed contains interacting keratinocyte, fibroblast, endothelial, and immune-cell programs, and these programs may differ between ulcers that close and those that remain chronically inflamed. Single-cell RNA sequencing has provided a direct view of this cellular heterogeneity,

including DFU-specific fibroblast and macrophage states described in the source GSE165816 study.

Clinical non-healing is unlikely to reflect one isolated molecular defect. Chronic ulcers often combine impaired re-epithelialization, altered matrix turnover, endothelial dysfunction, persistent or dysregulated inflammation, and metabolic stress within the same lesion [1,3–6]. Bulk-tissue studies have nominated cytokine, growth-factor, and extracellular-matrix pathways, but bulk profiles cannot determine whether a signal reflects a transcriptional state within a resident cell type or a shift in cellular composition [3,11–14]. This distinction matters when public transcriptomic data are used to nominate tractable markers for follow-up assays.

Single-cell RNA sequencing addresses this challenge by preserving cell-level information, yet clinical translation still requires specimen-level summaries because the patient or tissue sample remains the biological unit [15–19]. In DFU studies with modest patient numbers, uncritical cell-level testing can overstate precision. A specimen-centered pseudobulk design provides a more cautious bridge between rich single-cell data and the small-sample reality of public wound cohorts.

For a practical secondary-analysis manuscript, the most defensible approach is to treat each public specimen as the statistical unit rather than treating every cell as an independent biological replicate. We therefore reanalyzed GSE165816 raw count matrices using sample-level pseudobulk expression and marker signatures. This design prioritizes reproducible descriptive signals and avoids inflated significance that can arise when single cells are modeled as independent patient observations.

The objective of this analysis was to determine whether the public GSE165816 dataset could support an independent, concise manuscript focused specifically on non-healing DFU. We asked whether sample-level signatures and simple gene-level summaries could separate healing and non-healing DFU specimens, while keeping the claims appropriate for a secondary analysis without external validation.

2. Methods

2.1. Study design and data source

Raw count matrices from GSE165816 were obtained from the local project data directory. GEO sample annotations were used to identify tissue source and clinical disease group. The primary comparison used foot-skin specimens only, excluding PBMC and forearm samples from the main healing versus non-healing contrast.

The study was designed as a secondary analysis of a previously published public single-cell RNA-seq cohort [2,20]. No attempt was made to reclassify clinical outcomes beyond the public annotations. Samples annotated as healing DFU and non-healing DFU formed the primary contrast, whereas diabetic non-DFU and non-diabetic foot-skin samples were retained to describe the broader sample landscape and to help interpret whether DFU-associated signatures were directionally plausible.

2.2. Pseudobulk construction and normalization

For each sample, counts were summed by gene to produce a pseudobulk profile. Expression values were normalized as log2 counts per million plus one. Predefined marker signatures were scored by averaging normalized expression of genes representing fibroblast identity, inflammatory fibroblast activation, extracellular matrix remodeling, angiogenesis and endothelial activation, macrophage and monocyte states, keratinocyte activation, T-cell cytotoxicity, neutrophil activation, and hypoxia response.

This specimen-level summarization was selected to align the analysis with the number of independent biological samples rather than the number of captured cells. The approach sacrifices cell-subtype resolution but reduces the risk of pseudoreplication and produces tables that can be interpreted by laboratories planning low-cost validation experiments. Gene signatures were treated as descriptive summaries of biological programs, not as definitive cell-type abundance estimates.

2.3. Statistical analysis and marker prioritization

Differential expression between non-healing and healing DFU was calculated across sample-level pseudobulk profiles using Welch tests, followed by Benjamini-Hochberg adjustment. Because the analysis used a modest public dataset, genes were treated as candidates for prioritization rather than validated diagnostic markers. Apparent discrimination was summarized using rank-based AUC on the same dataset and was not presented as external validation.

Candidate markers were prioritized by combining direction of change, nominal statistical evidence, adjusted false-discovery estimates, and practical interpretability. A compact panel was then assembled from the top-ranked candidates to illustrate whether a small number of genes could summarize the healing versus non-healing contrast. The panel was not optimized through cross-validation because the number of independent DFU specimens was too small for a stable training-testing split.

All analyses used transparent tabular outputs and reproducible scripted figure generation. The figures emphasize sample composition, signature distributions, and gene-prioritization patterns because these outputs are the most suitable for an exploratory public-data manuscript. Reported *P*-values and AUC values should therefore be read as internal prioritization metrics rather than clinical diagnostic performance estimates.

3. Results

Figure 1 shows the overview of the conservative public single-cell RNA-seq secondary-analysis workflow.

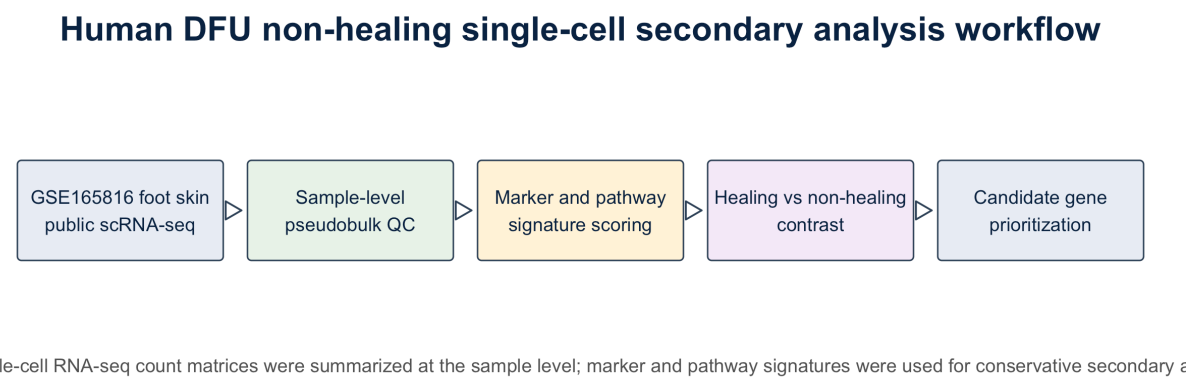

Figure 1. Overview of the conservative public single-cell RNA-seq secondary-analysis workflow

3.1. Foot-skin sample composition

The complete GSE165816 raw dataset contained 174,962 cells across 54 matrices. Restricting the principal analysis to foot skin yielded 94,325 cells from 33 specimens. This subset included non-diabetic, diabetic non-DFU, healing DFU, and non-healing DFU samples, allowing the non-healing contrast to be interpreted within the local foot-skin context. See Table 1 and Figure 2.

The first stage of analysis confirmed that the dataset contained enough independent foot-skin specimens to support a descriptive comparison, while also showing why the analysis needed to remain conservative. Healing and non-healing DFU were represented by fewer samples than the total cell count might suggest; thus, specimen-level aggregation provided a more realistic denominator for interpretation.

Table 1. Foot-skin GSE165816 sample composition used in the main analysis

Disease	Samples	Cells	Median_cells
DFU-healer	9	28,365	2,865
DFU-nonhealer	5	17,149	3,413
Non-DFU diabetic	8	12,723	1,567
Non-diabetic	11	36,088	3,465

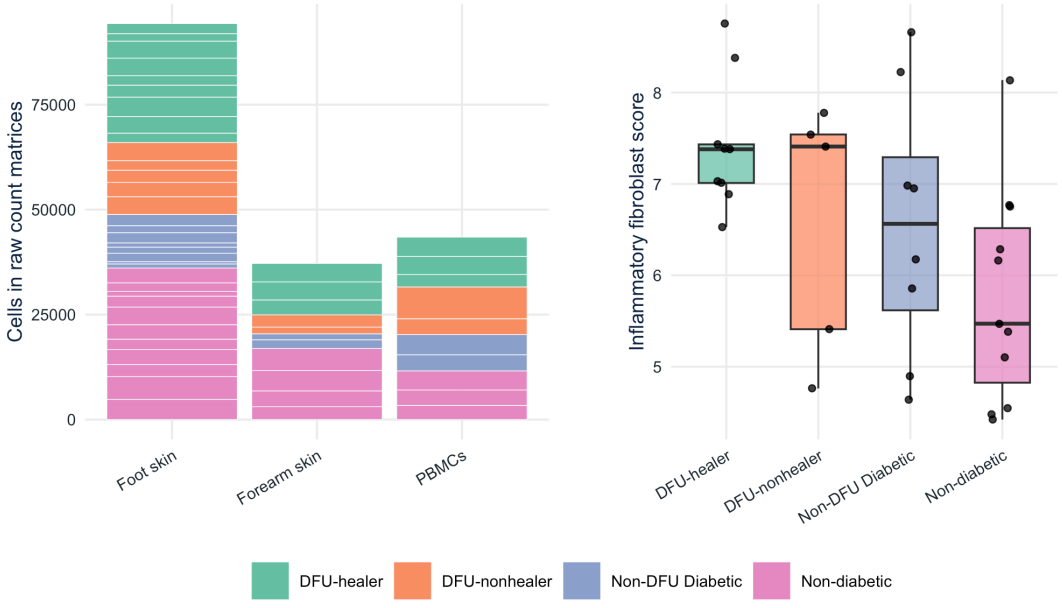

Figure 2. Sample landscape and inflammatory fibroblast marker-score distribution across clinical groups

3.2. Wound-repair signature landscape

Marker-score analysis suggested that healing and non-healing DFU samples differed in wound-repair-associated programs rather than in a single isolated pathway (Figure 3). Several inflammatory, keratinocyte, and matrix signatures were lower in non-healing samples than in healing DFU samples, consistent with the idea that productive repair requires organized inflammatory and stromal activation rather than simple absence of inflammation.

The signature landscape also indicated that diabetic non-DFU and non-diabetic foot-skin samples provided useful anchors for interpreting DFU-associated remodeling. Healing DFU specimens tended to show a more coordinated activation pattern across repair-related modules, whereas non-healing specimens displayed a less synchronized profile. This observation supports a model in which non-healing reflects disordered timing and coordination of repair programs rather than uniform suppression of every wound-response pathway.

Because signature scores were averaged from gene sets rather than inferred through deconvolution, they should not be interpreted as precise cell fractions. Their value lies in reducing thousands of genes into biologically interpretable wound processes, allowing the same specimens to be compared across fibroblast, keratinocyte, endothelial, and immune axes.

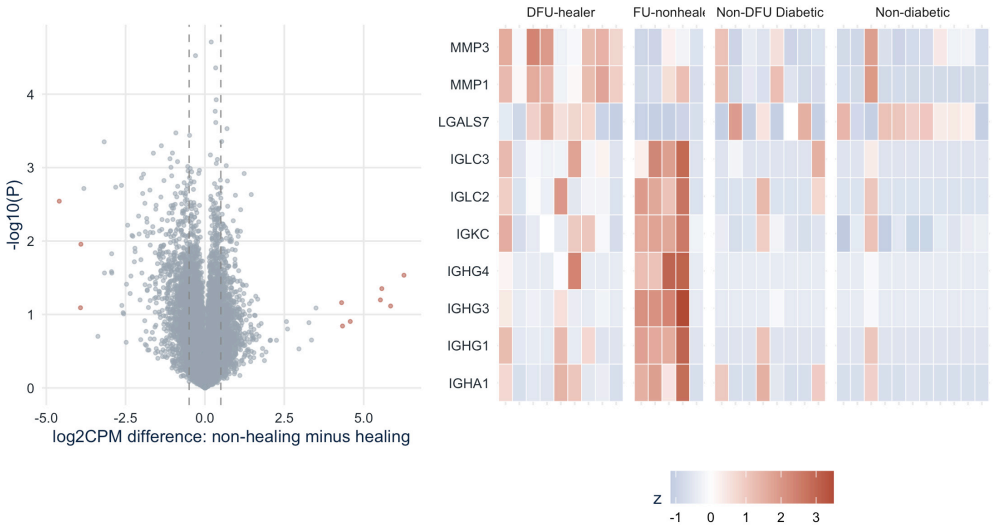

Figure 3. Sample-level gene prioritization for the non-healing versus healing DFU comparison

3.3. Candidate genes for non-healing DFU

The gene-level contrast prioritized a compact set of transcripts that separated non-healing from healing DFU in the internal sample-level analysis (Table 2). The resulting panel (IGHG3, IGLC2, IGKC, IGHG1, MMP3, IGHA1) achieved an apparent AUC of 0.89. The panel included immune-related immunoglobulin transcripts and matrix-remodeling genes, suggesting that both immune composition and stromal remodeling contribute to the sample-level separation.

The prioritized genes should be considered a practical starting point for validation rather than a final biomarker set. For example, immunoglobulin transcripts may reflect local B-cell or plasma-cell activity, local inflammation, or sample composition, whereas MMP3 is more directly interpretable as a matrix-remodeling transcript. A future validation study could test these genes by qPCR or immunostaining in independent DFU biopsies and examine whether their combination improves prediction beyond standard clinical variables.

Table 2. Data-driven candidate genes from the non-healing versus healing DFU pseudobulk contrast

Gene	LogFC	*P*_value	Adj_*P*_value	AUC_nonhealing	Included_in_panel
IGHG3	6.270	0.029	0.894	0.871	True
IGLC2	5.849	0.077	0.894	0.836	True
IGKC	5.572	0.044	0.894	0.943	True
IGHG1	5.527	0.063	0.894	0.868	True
MMP3	-4.592	0.003	0.846	0.646	True
IGHA1	4.578	0.124	0.894	0.804	True
IGLC3	4.334	0.144	0.894	0.836	False
IGHG4	4.305	0.069	0.894	0.868	False
MMP1	-3.923	0.081	0.894	0.507	False
LGALS7	-3.910	0.011	0.894	0.771	False

4. Discussion

4.1. Principal findings

This secondary analysis supports the feasibility of using public DFU single-cell matrices to nominate wound-state markers.

The strongest data-driven candidates included immunoglobulin-related transcripts and MMP3, indicating that sample-level immune-cell composition and stromal remodeling may contribute to the separation of healing and non-healing specimens. These results should be viewed as hypothesis-generating because the public cohort contains a limited number of independent non-healing DFU samples.

The study adds value by translating a complex single-cell resource into specimen-level outputs that are easier to compare with clinical validation designs. Rather than trying to reproduce every cell-type-specific finding of the source study, the manuscript asks a narrower question: whether non-healing DFU specimens carry detectable sample-level programs that can be summarized as signatures and candidate genes. That narrower framing is well-suited to a small independent paper.

4.2. Interpretation in relation to wound biology

The results are compatible with current views of DFU pathobiology in which chronicity arises from the interaction of inflammation, impaired matrix remodeling, angiogenic dysfunction, and defective epithelial repair [1,4–14]. The observation that healing DFU can show stronger organized wound-response signatures than non-healing DFU is biologically plausible: acute inflammation and matrix remodeling are necessary for repair, but they must be coordinated and resolved over time. A low or disordered signature may therefore represent an ineffective repair state rather than a healthier wound environment.

The presence of immunoglobulin-associated genes among candidate markers also deserves careful interpretation. These transcripts may signal adaptive immune infiltration, local antibody-producing cells, or broader inflammatory composition. They are not automatically specific biomarkers of non-healing tissue, but they may be useful as part of a combined panel if validated with histology, flow cytometry, or spatial transcriptomics.

4.3. Strengths and limitations

The main strength of the analysis is its conservative statistical framing. By summarizing cells into specimen-level pseudobulk profiles, the analysis avoids treating thousands of cells from the same patient as independent observations. The main limitation is that the workflow does not replace full cell-type-resolved integration, spatial validation, or external patient-level confirmation. The marker panel should therefore be considered a short list for qPCR, immunostaining, or prospective cohort validation rather than a ready clinical diagnostic model.

Additional limitations include reliance on public annotations, possible heterogeneity in biopsy site and ulcer duration, and the absence of harmonized clinical covariates such as infection status, vascular supply, treatment history, and glycemic control. The analysis also used predefined marker signatures, which improve interpretability but may miss unanticipated programs. Finally, the AUC was estimated in the same data used to nominate markers and should not be described as validated predictive performance.

5. Implications

Despite these limitations, the analysis provides a useful candidate list and a reproducible workflow for laboratories that want to mine public DFU single-cell data without overstating statistical certainty. The most immediate next step would be a modest independent cohort in which the panel genes and selected signature markers are measured in DFU biopsies with prospectively recorded healing outcomes. Such a study would determine whether the candidate signals are reproducible at the patient level and whether they add information to standard wound assessment.

Disclosure statement

The authors declare no conflict of interest.

References

[1] Armstrong DG, Boulton AJM, Bus SA, 2017, Diabetic Foot Ulcers and Their Recurrence. New England Journal of Medicine, 376(24): 2367–2375.

[2] Theocharidis G, Thomas BE, Sarkar D, et al., 2022, Single Cell Transcriptomic Landscape of Diabetic Foot Ulcers. Nature Communications, 13(1): 181.

[3] Theocharidis G, Baltzis D, Roustit M, et al., 2020, Integrated Skin Transcriptomics and Serum Multiplex Assays Reveal Novel Mechanisms of Wound Healing in Diabetic Foot Ulcers. Diabetes, 69(10): 2157–2169.

[4] Mohsin F, Javaid S, Tariq M, et al., 2024, Molecular Immunological Mechanisms of Impaired Wound Healing in Diabetic Foot Ulcers, Current Therapeutic Strategies and Future Directions. International Immunopharmacology, 139: 112713.

[5] Dawi J, Tumanyan K, Tomas K, et al., 2025, Diabetic Foot Ulcers: Pathophysiology, Immune Dysregulation, and Emerging Therapeutic Strategies. Biomedicines, 13(5): 1076.

[6] Wang J, Yang X, Zhou T, et al., 2025, Microenvironment of Diabetic Foot Ulcers: Implications for Healing and Therapeutic Strategies. Journal of Research in Medical Sciences, 30: 45.

[7] Song J, Zhao T, Wang C, et al., 2025, Cell Migration in Diabetic Wound Healing: Molecular Mechanisms and Therapeutic Strategies. International Journal of Molecular Medicine, 56(2): 103.

[8] Ru Y, Cai Y, Cheng G, et al., 2025, Immune Cells in Diabetic Wound Repair: The Key to Better Wound Management. Frontiers of Medicine.

[9] Zhang Y, Guo D, Song L, et al., 2026, Inflammation and Wound Healing: A Comprehensive Overview of Mechanisms, Therapeutic Strategies, and Translational Perspectives. Biomarker Research, 14: 57.

[10] Hu X, Dong D, Wang Y, et al., 2026, Integrative Bioinformatic Analysis Prioritizes TIMP1 and FN1 as Angiogenesis-Related Candidate Genes in Diabetic Foot Ulcers. PLoS One, 21(2): e0348808.

[11] Qing C, 2017, The Molecular Biology in Wound Healing and Non-Healing Wound. Chinese Journal of Traumatology, 20(4): 189–193.

[12] Barrientos S, Brem H, Stojadinovic O, et al., 2014, Clinical Application of Growth Factors and Cytokines in Wound Healing. Wound Repair and Regeneration, 22(5): 569–578.

[13] Marti-Carvajal AJ, Gluud C, Nicola S, et al., 2015, Growth Factors for Treating Diabetic Foot Ulcers. Cochrane Database of Systematic Reviews, (10): CD008548.

[14] den Dekker A, Davis FM, Kunkel SL, et al., 2019, Targeting Epigenetic Mechanisms in Diabetic Wound Healing. Translational Research, 204: 39–50.

[15] Stuart T, Butler A, Hoffman P, et al., 2019, Comprehensive Integration of Single-Cell Data. Cell, 177(7): 1888–1902.e21.

[16] Hao Y, Hao S, Andersen-Nissen E, et al., 2021, Integrated Analysis of Multimodal Single-Cell Data. Cell, 184(13): 3573–3587.e29.

[17] Crowell HL, Soneson C, Germain PL, et al., 2020, Muscat Detects Subpopulation-Specific State Transitions from Multi-Sample Multi-Condition Single-Cell Transcriptomics Data. Nature Communications, 11: 6077.

[18] Gilis J, Perin L, Malfait M, et al., 2025, Differential Detection Workflows for Multi-Sample Single-Cell RNA-seq Data. BMC Genomics, 26: 911.

[19] Hafner L, Sturm G, Lumpp S, et al., 2025, Single-Cell Differential Expression Analysis Between Conditions Within Nested Settings. Briefings in Bioinformatics, 26(4): bbaf397.

[20] Theocharidis G, Thomas BE, Sarkar D, et al., 2022, Single Cell Transcriptomic Landscape of Diabetic Foot Ulcers. Nat Commun, 13(1): 181.

Publisher's note

Whioce Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.