INOSR Scientific Research 12(3):1-4, 2025.
©INOSR PUBLICATIONS
International Network Organization for Scientific Research
https://doi.org/10.59298/INOSRSR/2025/1231400

ISSN: 2705-1706
INOSRSR12300000

Single-Cell RNA Sequencing Reveals Heterogeneity in Immune-Stromal Crosstalk within Diabetic Foot Ulcers: Toward Precision Wound Healing

Kamanzi Ntakirutimana G.

School of Natural and Applied Sciences Kampala International University Uganda

ABSTRACT

Diabetic foot ulcers (DFUs) are a significant complication of diabetes mellitus, characterized by chronic inflammation, impaired angiogenesis, and dysfunctional immune-stromal interactions that hinder wound healing. Despite advancements in conventional wound management, DFUs remain challenging to treat due to their cellular heterogeneity. Single-cell RNA sequencing (scRNA-seq) has emerged as a transformative tool in elucidating the complexity of DFU pathophysiology by providing high-resolution insights into the immune and stromal cellular landscape. This review, written using a narrative synthesis approach, explored how scRNA-seq has uncovered distinct immune cell subsets, including pro-inflammatory macrophages, persistent neutrophils, and dysregulated T and B cell populations, as well as stromal cell dysfunction affecting fibroblasts, endothelial cells, and pericytes. Furthermore, scRNA-seq has shed light on aberrant cytokine signaling, extracellular matrix remodeling, and angiogenic deficiencies that contribute to chronic wound pathology. The integration of these findings into therapeutic strategies highlights the potential for precision medicine approaches, including targeted immunomodulation, pro-angiogenic therapies, and fibroblast-based interventions tailored to individual patient profiles. By leveraging scRNA-seq data, future research can refine personalized wound healing protocols, ultimately improving outcomes for DFU patients. This review underscored the need for further investigations into single-cell technologies to enhance the development of novel and effective therapeutic interventions.

Keywords: Single-cell RNA sequencing (scRNA-seq), Diabetic foot ulcers (DFUs), Immune-stromal crosstalk, Precision wound healing, Cellular heterogeneity.

INTRODUCTION

Diabetic foot ulcers (DFUs) represent a major complication of diabetes mellitus, often leading to chronic non-healing wounds, severe infections, and limb amputations [1, 2]. Despite advances in wound management, DFUs remain a significant healthcare burden, with limited therapeutic options tailored to individual patients. The chronic nature of DFUs is attributed to a dysregulated wound-healing process, driven by persistent inflammation, impaired angiogenesis, and dysfunctional cellular interactions [3, 4]. Conventional histological and bulk transcriptomic approaches have provided insights into the pathophysiology of DFUs, but they fail to capture the cellular heterogeneity and complex immune-stromal interactions within the wound microenvironment. Single-cell RNA sequencing (scRNA-seq) has emerged as a powerful tool to dissect the molecular landscape of DFUs at an unprecedented resolution [5, 6]. By enabling the characterization of individual cells within the wound milieu, scRNA-seq allows for the identification of distinct immune and stromal cell subsets, their dynamic interactions, and their roles in impaired wound healing [7]. This review explores the application of scRNA-seq in elucidating immune-stromal crosstalk within DFUs, highlighting key findings, therapeutic implications, and future research directions. Understanding these intricate cellular interactions may pave the way for precision wound healing strategies, ultimately improving patient outcomes.

Cellular Heterogeneity in Diabetic Foot Ulcers: Insights from scRNA-seq

scRNA-seq has revealed a complex cellular ecosystem within DFUs, characterized by diverse immune and stromal cell populations with distinct functional states [8]. These cells play critical roles in the inflammatory response, extracellular matrix remodeling, and tissue repair. The Immune Cell Subsets and Their Roles in DFUs include:

i. **Macrophages:** Macrophages exhibit plasticity, transitioning between pro-inflammatory (M1) and anti-inflammatory (M2) states [9]. In chronic DFUs, scRNA-seq analyses have identified an overrepresentation of pro-inflammatory macrophages, contributing to sustained inflammation and delayed healing. M1

macrophages produce high levels of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), exacerbating tissue damage and impairing re-epithelialization. Conversely, M2 macrophages, which promote tissue repair through the secretion of transforming growth factor-beta (TGF-β) and vascular endothelial growth factor (VEGF), are often underrepresented in chronic wounds.

ii. **Neutrophils:** Neutrophils are essential for the early stage of wound healing, providing antimicrobial defense and recruiting other immune cells [10]. However, scRNA-seq has identified persistent neutrophil infiltration in DFUs, leading to excessive release of reactive oxygen species (ROS) and neutrophil extracellular traps (NETs), which further damage the surrounding tissue and sustain the inflammatory phase.

iii. **T Cells and B Cells:** Dysregulated adaptive immune responses have also been implicated in DFU pathogenesis. scRNA-seq studies have demonstrated an imbalance in T cell subsets, with elevated levels of pro-inflammatory Th1 and Th17 cells and a deficiency in regulatory T cells (Tregs), which are critical for resolving inflammation [11, 12]. Additionally, B cell populations in DFUs exhibit aberrant activation, contributing to chronic inflammation through autoantibody production and cytokine secretion.

Stromal Cell Populations and Their Interactions

i. **Fibroblasts:** Fibroblasts are essential for extracellular matrix (ECM) deposition and wound closure [13]. scRNA-seq has identified distinct fibroblast subpopulations in DFUs, including pro-inflammatory fibroblasts that secrete IL-1β and chemokines, contributing to prolonged inflammation. Conversely, the population of pro-reparative fibroblasts, which promote ECM synthesis and collagen deposition, is often diminished.

ii. **Endothelial Cells:** Angiogenesis is a crucial process for wound healing, but it is impaired in DFUs. scRNA-seq has revealed dysfunctional endothelial cell subtypes within chronic wounds, characterized by reduced expression of angiogenic factors such as VEGF and increased markers of endothelial senescence and apoptosis. These findings suggest that impaired vascular remodeling contributes to hypoxia and delayed healing in DFUs.

iii. **Pericytes and Myofibroblasts:** Pericytes play a vital role in vascular stability, while myofibroblasts are key effectors of wound contraction [14]. scRNA-seq has uncovered altered pericyte-to-myofibroblast transition dynamics in DFUs, with defective myofibroblast activation leading to impaired wound contraction and ECM remodeling.

Immune-Stromal Crosstalk in DFUs

The interactions between immune and stromal cells are critical for orchestrating the wound-healing response. scRNA-seq has provided novel insights into these interactions, revealing aberrant signaling networks that contribute to chronic wound pathology.

i. **Cytokine and Chemokine Signaling:** Chronic DFUs exhibit dysregulated cytokine and chemokine profiles, with excessive pro-inflammatory signaling between immune and stromal cells. Elevated levels of IL-1β, IL-6, and TNF-α perpetuate inflammation, while decreased anti-inflammatory cytokines such as IL-10 and TGF-β hinder resolution and tissue repair [15].

ii. **Extracellular Matrix Remodeling:** The ECM serves as a structural scaffold for tissue regeneration. scRNA-seq has revealed that immune cells, particularly macrophages and fibroblasts, engage in aberrant ECM remodeling, leading to excessive fibrosis or ECM degradation [16]. Dysregulated matrix metalloproteinase (MMP) activity further disrupts ECM homeostasis, contributing to chronic wound persistence.

iii. **Hypoxia and Angiogenic Dysfunction:** Hypoxia is a hallmark of chronic DFUs, exacerbating immune-stromal dysfunction. scRNA-seq studies have identified impaired hypoxia-inducible factor (HIF) signaling in endothelial cells and fibroblasts, resulting in insufficient VEGF production and poor neovascularization. This vascular insufficiency prolongs tissue hypoxia, further hindering wound closure.

Therapeutic Implications and Precision Medicine Approaches

The insights gained from scRNA-seq analyses have significant implications for developing targeted therapies and precision medicine strategies for DFUs.

i. **Modulating Macrophage Polarization:** Therapeutic strategies aimed at shifting macrophage phenotypes from pro-inflammatory to reparative states hold promises for improving wound healing. Nanoparticle-based delivery of anti-inflammatory agents, such as IL-4 and IL-10, has shown potential in restoring macrophage homeostasis [17, 18].

ii. **Targeting Neutrophil Dysfunction:** Strategies to modulate neutrophil lifespan and function, such as inhibitors of NET formation or ROS scavengers, may mitigate excessive neutrophil-mediated tissue damage [19].

iii. **Enhancing Angiogenesis:** Pro-angiogenic therapies, including VEGF-mimetic peptides, endothelial progenitor cell therapies, and gene editing approaches targeting HIF pathways, may restore vascular integrity in DFUs [20].

iv. **Fibroblast-Based Therapies:** Novel fibroblast-based interventions, such as autologous fibroblast transplantation or fibroblast-derived extracellular vesicles, may promote ECM remodeling and wound closure.

v. **Personalized Wound Care Strategies:** The heterogeneity revealed by scRNA-seq underscores the need for personalized treatment approaches tailored to individual wound profiles. Machine learning algorithms integrating scRNA-seq data with clinical parameters may facilitate the development of precision wound care protocols [21].

CONCLUSION

Single-cell RNA sequencing has revolutionized our understanding of the cellular and molecular landscape of diabetic foot ulcers, uncovering previously unrecognized heterogeneity in immune-stromal crosstalk. The identification of distinct immune and stromal cell subsets, their functional states, and their dysregulated interactions provides a foundation for developing targeted therapies aimed at restoring wound-healing homeostasis. Precision medicine approaches informed by scRNA-seq data hold promise for overcoming the challenges associated with chronic DFUs, ultimately leading to improved clinical outcomes. Future research should focus on translating these findings into therapeutic interventions and integrating multi-omic approaches to further refine personalized treatment strategies. By harnessing the power of single-cell technologies, we can move toward more effective and individualized wound healing solutions for patients suffering from diabetic foot ulcers.

REFERENCES

1. Baig, M.S., Banu, A., Zehravi, M., Rana, R., Burle, S.S., Khan, S.L., Islam, F., Siddiqui, F.A., Massoud, E.E.S., Rahman, M.H., Cavalu, S.: An Overview of Diabetic Foot Ulcers and Associated Problems with Special Emphasis on Treatments with Antimicrobials. Life 2022, Vol. 12, Page 1054. 12, 1054 (2022). https://doi.org/10.3390/LIFE12071054
2. Mohsin, F., Javaid, S., Tariq, M., Mustafa, M.: Molecular immunological mechanisms of impaired wound healing in diabetic foot ulcers (DFU), current therapeutic strategies and future directions. Int Immunopharmacol. 139, 112713 (2024). https://doi.org/10.1016/J.INTIMP.2024.112713
3. Mohsin, F., Javaid, S., Tariq, M., Mustafa, M.: Molecular immunological mechanisms of impaired wound healing in diabetic foot ulcers (DFU), current therapeutic strategies and future directions. Int Immunopharmacol. 139, 112713 (2024). https://doi.org/10.1016/J.INTIMP.2024.112713
4. Davis, F.M., Kimball, A., Boniakowski, A., Gallagher, K.: Dysfunctional Wound Healing in Diabetic Foot Ulcers: New Crossroads. Curr Diab Rep. 18, 1–8 (2018). https://doi.org/10.1007/S11892-018-0970-Z/METRICS
5. Chen, R., Zou, L.: Combined analysis of single-cell sequencing and bulk transcriptome sequencing reveals new mechanisms for non-healing diabetic foot ulcers. PLoS One. 19, e0306248 (2024). https://doi.org/10.1371/JOURNAL.PONE.0306248
6. Dong, Y., Wang, M., Wang, Q., Cao, X., Chen, P., Gong, Z.: Single-cell RNA-seq in diabetic foot ulcer wound healing. Wound Repair and Regeneration. 32, 880–889 (2024). https://doi.org/10.1111/WRR.13218;WEBSITE:WEBSITE:PERICLES;JOURNAL:JOURNAL:1524475X;WGROUP:STRING:PUBLICATION
7. Dong, Y., Wang, M., Wang, Q., Cao, X., Chen, P., Gong, Z.: Single-cell RNA-seq in diabetic foot ulcer wound healing. Wound Repair and Regeneration. 32, 880–889 (2024). https://doi.org/10.1111/WRR.13218;WEBSITE:WEBSITE:PERICLES;JOURNAL:JOURNAL:1524475X;WGROUP:STRING:PUBLICATION
8. Li, Y., Ju, S., Li, X., Li, W., Zhou, S., Wang, G., Cai, Y., Dong, Z.: Characterization of the microenvironment of diabetic foot ulcers and potential drug identification based on scRNA-seq. Front Endocrinol (Lausanne). 13, 997880 (2023). https://doi.org/10.3389/FENDO.2022.997880/BIBTEX
9. Shapouri-Moghaddam, A., Mohammadian, S., Vazini, H., Taghadosi, M., Esmaeili, S.A., Mardani, F., Seifi, B., Mohammadi, A., Afshari, J.T., Sahebkar, A.: Macrophage plasticity, polarization, and function in health and disease. J Cell Physiol. 233, 6425–6440 (2018). https://doi.org/10.1002/JCP.26429
10. Phillipson, M., Kubes, P.: The Healing Power of Neutrophils. Trends Immunol. 40, 635–647 (2019). https://doi.org/10.1016/J.IT.2019.05.001/ASSET/0E99A0C9-3A9F-4FCE-82C0-757AFC9BF8EC/MAIN.ASSETS/GR2.SML

11. Yuanyuanxu, Qipeng, Qingqingma, Yao, X.: scRNA + TCR-seq revealed the dual TCR pTh17 and Treg T cells involvement in autoimmune response in ankylosing spondylitis. Int Immunopharmacol. 135, 112279 (2024). https://doi.org/10.1016/J.INTIMP.2024.112279
12. Obeagu, E.I., Alum, E.U., Ugwu, O.P.C.: Hepcidin's Antimalarial Arsenal: Safeguarding the Host. Newport International Journal Of Public Health And Pharmacy. 4, 1–8 (2023). https://doi.org/10.59298/NIJPP/2023/10.1.1100
13. Diller, R.B., Tabor, A.J.: The Role of the Extracellular Matrix (ECM) in Wound Healing: A Review. Biomimetics 2022, Vol. 7, Page 87. 7, 87 (2022). https://doi.org/10.3390/BIOMIMETICS7030087
14. Thomas, H.M., Cowin, A.J., Mills, S.J.: The Importance of Pericytes in Healing: Wounds and other Pathologies. International Journal of Molecular Sciences 2017, Vol. 18, Page 1129. 18, 1129 (2017). https://doi.org/10.3390/IJMS18061129
15. Obeagu, E.I., Obeagu, G.U., Alum, E.U., Ugwu, O.P.-C.: Persistent Immune Activation and Chronic Inflammation: Unraveling Their Impact on Anemia in HIV Infection. INOSR Experimental Sciences. 12, 73–84 (2023). https://doi.org/10.59298/INOSRES/2023/7.3.21322
16. Qian, W., Xia, S., Yang, X., Yu, J., Guo, B., Lin, Z., Wei, R., Mao, M., Zhang, Z., Zhao, G., Bai, J., Han, Q., Wang, Z., Luo, Q.: Complex Involvement of the Extracellular Matrix, Immune Effect, and Lipid Metabolism in the Development of Idiopathic Pulmonary Fibrosis. Front Mol Biosci. 8, 800747 (2022). https://doi.org/10.3389/FMOLB.2021.800747/BIBTEX
17. Wang, H., Zhou, Y., Sun, Q., Zhou, C., Hu, S., Lenahan, C., Xu, W., Deng, Y., Li, G., Tao, S.: Update on Nanoparticle-Based Drug Delivery System for Anti-inflammatory Treatment. Front Bioeng Biotechnol. 9, 630352 (2021). https://doi.org/10.3389/FBIOE.2021.630352/BIBTEX
18. Ahamad, N., Kar, A., Mehta, S., Dewani, M., Ravichandran, V., Bhardwaj, P., Sharma, S., Banerjee, R.: Immunomodulatory nanosystems for treating inflammatory diseases. Biomaterials. 274, 120875 (2021). https://doi.org/10.1016/J.BIOMATERIALS.2021.120875
19. Rizo-Téllez, S.A., Sekheri, M., Filep, J.G.: Myeloperoxidase: Regulation of Neutrophil Function and Target for Therapy. Antioxidants 2022, Vol. 11, Page 2302. 11, 2302 (2022). https://doi.org/10.3390/ANTIOX11112302
20. De Rosa, L., Di Stasi, R., D'Andrea, L.D.: Pro-angiogenic peptides in biomedicine. Arch Biochem Biophys. 660, 72–86 (2018). https://doi.org/10.1016/J.ABB.2018.10.01.
21. Yu, X., Wu, Z., Zhang, N.: Machine learning-driven discovery of novel therapeutic targets in diabetic foot ulcers. Molecular Medicine. 30, 1–15 (2024). https://doi.org/10.1186/S10020-024-00955-Z/METRICS

CITE AS: Kamanzi Ntakirutimana G. (2025). Single-Cell RNA Sequencing Reveals Heterogeneity in Immune-Stromal Crosstalk within Diabetic Foot Ulcers: Toward Precision Wound Healing. INOSR Scientific Research 12(3):1-4. https://doi.org/10.59298/INOSRSR/2025/1231400