# Towards a multiscale synthesis of skin regeneration: Single cell transcriptomics and modeling reveal functional fibroblast heterogeneity in skin wounds

[1]Christian Fernando Guerrero-Juarez*, [1]Maksim V. Plikus and [1]Qing Nie
[1]NSF-Simons Center for Multiscale Cell Fate Research, University of California, Irvine, Irvine, CA, US
email: cfguerre@uci.edu, website: http://nielab.math.uci.edu/people/christian-guerrero-juarez

**BACKGROUND:** During healing of large excisional skin wounds in adult mice, hair follicles and then dermal adipocytes regenerate anew in the wound center. Neogenic hair follicles regenerate from wound epidermis and wound fibroblasts by reactivating embryonic-like hair follicle morphogenesis programs. De novo adipocytes regenerate around newly formed hair follicles from myofibroblasts, a specialized contractile wound fibroblast, via the process of cellular reprogramming. We studied diversity of fibroblasts in large skin wounds using 3'-end single-cell RNA-sequencing. We demonstrate a large degree of fibroblast heterogeneity, as fibroblasts group into twelve distinct populations. Pseudotime and RNA velocity analyses demonstrates that some fibroblast clusters likely represent sequential states during fibroblast differentiation toward a contractile state, while other clusters appear to represent distinct fibroblast lineages. Through the implementation of a novel 2D, multiscale hybrid mathematical model, we demonstrate that skin fibroblasts determine wound outcome, and that fibroblast heterogeneity and their responses to specific wound signals determine final wound outcome. We demonstrate that a subset of wound fibroblasts expresses hematopoietic markers, suggesting a potential myeloid origin. We validated this finding using single-cell western blot as well as full-length single-cell RNA-sequencing on genetically labeled wound fibroblasts. Furthermore, using bone marrow transplantation and Cre recombinase-based lineage tracing experiments, we rule out cell fusion events and confirm that hematopoietic lineage cells give rise to a subset of wound fibroblasts that form new wound adipocytes. These findings suggest potential new treatments for skin wound healing and other fibrotic disorders.

**METHODS:** Large skin wounds were generated in the dorsum of adult mice. 12 days after wounding, mouse wound tissues were collected and subjected to droplet enabled 3'-end single cell RNA-sequencing. Sequencing data was analyzed bioinformatically using generic and custom R/Matlab/Python scripts. Wounding was modeled using a 2D, multiscale hybrid model (MHM) with submodel allocation on two distinct wound compartments. Tissue immunostaining was performed on frozen skin wound tissues to spatially define fibroblast subtypes. Single cell Western blot was performed in tdTomato-labeled fibroblasts to confirm co-expression of myeloid markers. Bone marrow transplantation assays were performed in lethally irradiated mice with GFP-labeled adipose-specific Cre hematopoetic stem cells. Lineage tracing of myeloid cells using pan-myeloid marker Cre line demonstrated a myeloid-origin of fibroblasts and newly regenerated wound adipocytes.

**RESULTS:** Dimensionality reduction of single cell data onto t-SNE embedding shows distinct fibroblast subtype clustering in 12-day old skin wounds (**Figure 1**). Hierarchical clustering of these sub-types identified that they clustered mainly in two main groups, *Rgs5*$^{+}$ and *Pdgfra*$^{+}$ cells, with the latter subdividing further into *Mest*$^{+}$ and *Crabp*$^{+}$ subtypes. 2D HMH revealed that fibroblast response to wound environment, coupled with wound geometry, predicts skin wound healing outcomes (**Figure 2A**). Lineage tracing of myeloid cells using *LysM-Cre* mice shows that myeloid-derived fibroblasts regenerate wound adipocytes (**Figure 2B**).

**CONCLUSIONS:** Herein we demonstrate that wounding in skin induces a high degree of heterogeneity among fibroblasts and suggests that at least two main fibroblast lineages co-exist in large skin wounds, with one deriving from myeloid cells that contribute to skin regeneration. Mathematical modeling of skin wounds predicts a functional role of fibroblasts on wound.

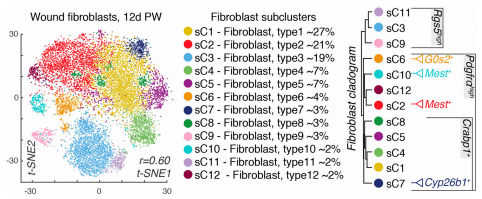


**Figure 1**: Single cell RNA-sequencing identifies twelve distinct fibroblast subtypes in large skin wounds. tSNE embedding shows distinct fibroblast subtypes. The proportions of fibroblast subtypes are on the right and color coded. Hierarchical clustering shows relationships between fibroblast subtypes and their lineages.

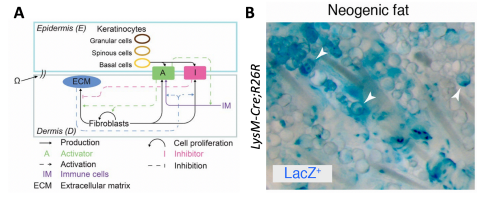


**Figure 2**: (**A**) Schematic of 2D multiscale hybrid model of wound healing. (**B**) Lineage tracing of newly regenerated adipocytes (blue/LacZ$^{+}$).

**REFERENCES:**
1. Guerrero-Juarez, *et al*. *Nat Commun*. 2019. 10(1):650.
2. Wang, *et al*. *Exp Dermatol*. 2019. (4):493-502.

**ACKNOWLEDGEMENTS:** This work is supported by NIH grant U01-AR073159 (to Q.N., M.V.P., and Xing Dai) and NSF-Simons Center grants NSF (DMS1763272, Q.N.) and Simons Foundation grant (594598, Q.N.)