JEM Journal of Experimental Medicine

INSIGHTS

Fast and scarless: Prx1⁺ fibroblasts turbocharge healing

Rachel M. Kratofil[1] and Shruti Naik[2]

Our oral cavity has evolved a capacity for rapid healing without scarring. In this issue of *JEM*, Ko et al. (2022. *J. Exp. Med.* https://doi.org/10.1084/jem.20221350) identify a Prx1⁺ fibroblast progenitor that drives oral regeneration by summoning pro-healing TGFβ1⁺ macrophages.

The epithelial barriers that line our body have evolved sophisticated repair mechanisms that limit penetration of disease-causing entities. Rapid repair after injury is particularly essential to cope with recurrent damage, such as that incurred during the process of chewing our food. Fibrosis or scarring has long been thought of as an evolutionary adaptive measure to address this "need for speed" (Gurtner et al., 2008). Surprisingly, however, the oral mucosa does not repair on canonical fibrosis, but nevertheless heals quickly without scarring (Häkkinen et al., 2000). Comparative studies between regenerative oral and scarring skin injury responses highlight the inflammatory responses as a key distinguisher of fibrotic repair (Iglesias-Bartolome et al., 2018). Yet, the reasons why skin may be more inflammatory, and the mechanisms involved in promoting regeneration in oral wounds, remained elusive.

In this study, Ko et al. (2022) delve into the mechanisms underlying oral regeneration by comparing injury responses in the anterior region of the palate (rugae 2, or R2), which heals rapidly, to posterior palate (R4) wounds that are slow to heal (see figure). In doing so, they identify a unique paired-related homeobox-1⁺ (Prx1⁺) fibroblast progenitor population in the R2 palate that is missing from the R4 palate. Depletion of Prx1⁺ fibroblasts delayed healing the R2 region, and conversely, transplanting these cells into the R4 region expedited repair, underscoring the importance of this population in driving divergent healing outcomes.

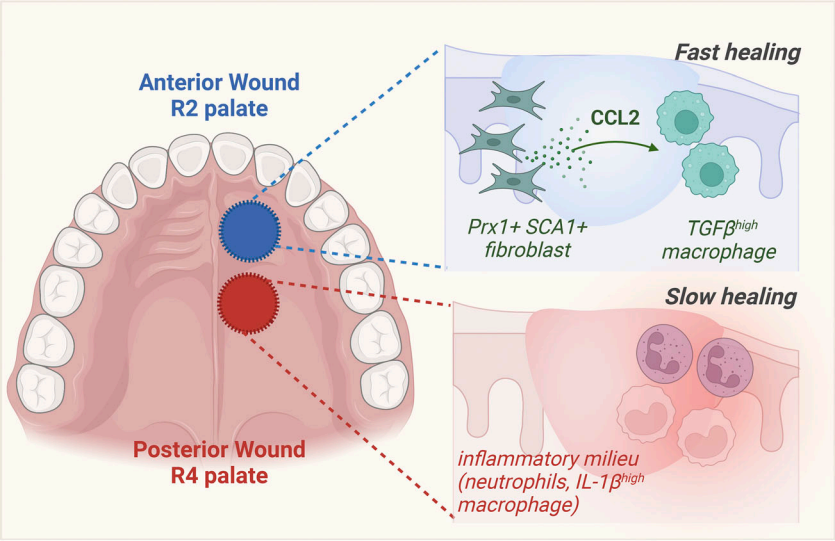
Next, the authors employed single-cell RNA-sequencing and lineage tracing to reveal that the Prx1⁺ cells were progenitor cells that differentiated into predominantly stem cell antigen-1 (SCA1⁺) fibroblasts following oral wounding. Gene ontology analyses identified these progenitor cells to have transcriptomic signatures involved in ossification, wound healing, and the Wnt signaling pathway. In addition, the SCA1⁺ fibroblasts expressed genes involved in immune cell chemotaxis including *Ccl2*.

CCL2, a chemoattractant that recruits monocytes and macrophages, was highly expressed by R2 wound SCA1⁺ fibroblasts. Accordingly, immune profiling revealed an enrichment in F4/80⁺ macrophages in anterior (R2) oral wounds. By contrast, the R4 wounds, which lacked Prx1⁺ fibroblasts, had more neutrophils. Functionally, R2 wound macrophages had a "pro-resolving" phenotype based on their expression of TGFβ1, while the small frequency of R4 wound macrophages expressed a pro-inflammatory phenotype characterized by higher expression of IL-1β. Depletion of Prx1⁺ cells in the oral mucosa caused R2 wounds to adopt an "R4-like" state with a neutrophilic milieu and a higher proportion of macrophages expressing IL-1β and fewer macrophages expressing TGFβ1. To determine if CCL2 is required for recruitment of pro-resolving macrophages, the authors generated $Prx1^{CreERT}$:$Ccl2^{f/f}$ mice to deplete CCL2 specifically in Prx1⁺ cells ($\Delta CCL2^{Prx1}$). Wounding the R2 region of $\Delta CCL2^{Prx1}$ mice delayed healing. Moreover, these wounds had fewer F4/80⁺ macrophages, a higher proportion of which had a proinflammatory phenotype and expressed IL-1β⁺ compared to CCL2 expressing controls. Whether this difference in immune cell recruitment is due to an increase in total numbers of macrophages recruited to R2 regions, or whether F4/80⁺ macrophages arise from circulating monocytes or tissue-resident macrophages remains to be tested. In addition to recruiting macrophages, how Prx1⁺ cells favor their

Insights from Rachel M. Kratofil and Shruti Naik.

[1]Department of Microbiology, NYU Langone Health, New York, NY, USA; [2]Department of Pathology, Department of Medicine, Ronald O. Perelman Department of Dermatology, and Perlmutter Cancer Center, NYU Langone Health, New York, NY, USA.

Shruti Naik: shruti.naik@nyulangone.org; Rachel M. Kratofil: rachel.kratofil@nyulangone.org.



Prx1⁺ fibroblasts expedite oral repair. Wounds in the anterior oral mucosa heal faster than wounds in the posterior mucosa. Prx1⁺ SCA1⁺ fibroblasts in the anterior rugae augment repair by recruiting pro-resolving macrophages in a CCL2-dependent manner. By contrast, the posterior wounds are enriched for inflammatory IL-1β⁺ macrophages and neutrophils. Figure created using Biorender.com.

pro-repair phenotype also warrants further investigation.

The authors tested whether the Prx1 cell–mediated healing can be transferred to the skin, which heals by scarring rather than regeneration. To do so, they adoptively transferred Prx1⁺ cells into the skin prior to wounding. Remarkably, adoptive transfer of Prx1⁺ cells was sufficient to accelerate wound closure in the skin, curbed the scarring response, and promoted regeneration of hair follicles. Finally, the authors identified Prx1⁺ fibroblasts within human oral mucosa, predominantly in the anterior palate, and these cells were also highly chemotactic, expressing *CCL2* and *CXCL1* transcripts. The ability to transfer the regenerative powers of the oral mucosa to other epithelial boundaries simply by transplanting Prx1⁺ cells suggested that these cells may aid the development of potent regenerative therapies for indications across all our body's barriers.

Prx1, a transcription factor and marker of mesenchymal stem cells, plays a role in bone development as well as bone regeneration after fracture (Duchamp de Lageneste et al., 2018; Martin and Olson, 2000). The precise gene expression programs controlled by this transcription factor in the context of repair, however, remained to be defined. Prx1⁺ fibroblast ablation in this study resulted in a more severe delay in healing than specific deletion of CCL2 in these cells, indicating that other Prx1 controlled healing mechanisms act in concert with immune modulatory functions.

This study opens the door to new questions regarding the origins of Prx1⁺ progenitors within the oral mucosa. How are these progenitors seeded within the oral niche, and are there tissue-specific cues that are necessary to retain these progenitors within the anterior region of the oral mucosa? Why are these cells only present in the R2 mucosa? Just as stromal cells are well known to modulate immune survival, localization, and function (Bonnardel et al., 2019), so too are fibroblasts themselves sensitive to tissue-specific imprinting and adopt unique functions based on their location (Buechler et al., 2021). Decoding the microenvironmental signals that direct formation of these cells could thus shed light on how to induce Prx1 progenitors or reprogram existing pathologic fibroblasts to adopt this functional phenotype.

Fibroblast heterogeneity in fibrotic repair versus scarless regeneration is an area of increasing intrigue. Specific anatomical locations as well as environmental triggers are known to contribute to the diversity of fibroblast fate and function (Talbott et al., 2022). For example, following full-thickness skin wounding, mechanical cues from the tissue environment can trigger activation of Yes-associated protein (YAP) specifically in Engrailed-1 expressing fibroblasts, and YAP inhibition is sufficient to drive regeneration (Mascharak et al., 2021). How mechanosensing and YAP signaling impacts the function of Prx1⁺ cells is yet to be determined. Distinct fibroblast subsets that reside within reindeer velvet also contribute to regenerative healing, where velvet fibroblasts adopt a fetal-like signature when compared to back skin fibroblasts that form scars (Sinha et al., 2022). Similar to the findings presented by Graves and colleagues, site-specific recruitment of immune cells that adopt a pro-resolving phenotype and limit overt inflammation underlies reindeer velvet regeneration (Sinha et al., 2022). The dynamic crosstalk between fibroblasts and macrophages fueling divergent repair outcomes is thus emerging as a theme in regenerative medicine across different tissue types and in different organisms.

Preventing scar formation and restoring the tissue's original structure and function via regeneration is an attractive goal for researchers and clinicians, as wound healing remains a major health care challenge with limited options for treatment. Thus, the findings from Ko et al. (2022) offer a promising therapeutic approach for the treatment of chronic wounds and other non-healing conditions that are underwritten by aberrant inflammation.

References

Bonnardel, J., et al. 2019. *Immunity*. https://doi.org/10.1016/j.immuni.2019.08.017
Buechler, M.B., et al. 2021. *Nature*. https://doi.org/10.1038/s41586-021-03549-5
Duchamp de Lageneste, O., et al. 2018. *Nat. Commun.* https://doi.org/10.1038/s41467-018-03124-z
Gurtner, G.C., et al. 2008. *Nature*. https://doi.org/10.1038/nature07039
Häkkinen, L., et al. 2000. *Periodontol. 2000*. 24: 127–152.
Iglesias-Bartolome, R., et al. 2018. *Sci. Transl. Med.* https://doi.org/10.1126/scitranslmed.aap8798
Ko, K.I., et al. 2022. *J. Exp. Med.* https://doi.org/10.1084/jem.20221350
Martin, J.F., and E.N. Olson. 2000. *Genesis*. https://doi.org/10.1002/(SICI)1526-968X(200004)26:4<225::AID-GENE10>3.0.CO;2-F
Mascharak, S., et al. 2021. *Science*. https://doi.org/10.1126/science.aba2374
Sinha, S., et al. 2022. *Cell*. https://doi.org/10.1016/j.cell.2022.11.004
Talbott, H.E., et al. 2022. *Cell Stem Cell*. https://doi.org/10.1016/j.stem.2022.07.006