THE ORAL MUCOSA IS ENRICHED WITH INFLAMMATORY FIBROBLASTS IN DIABETIC MICE

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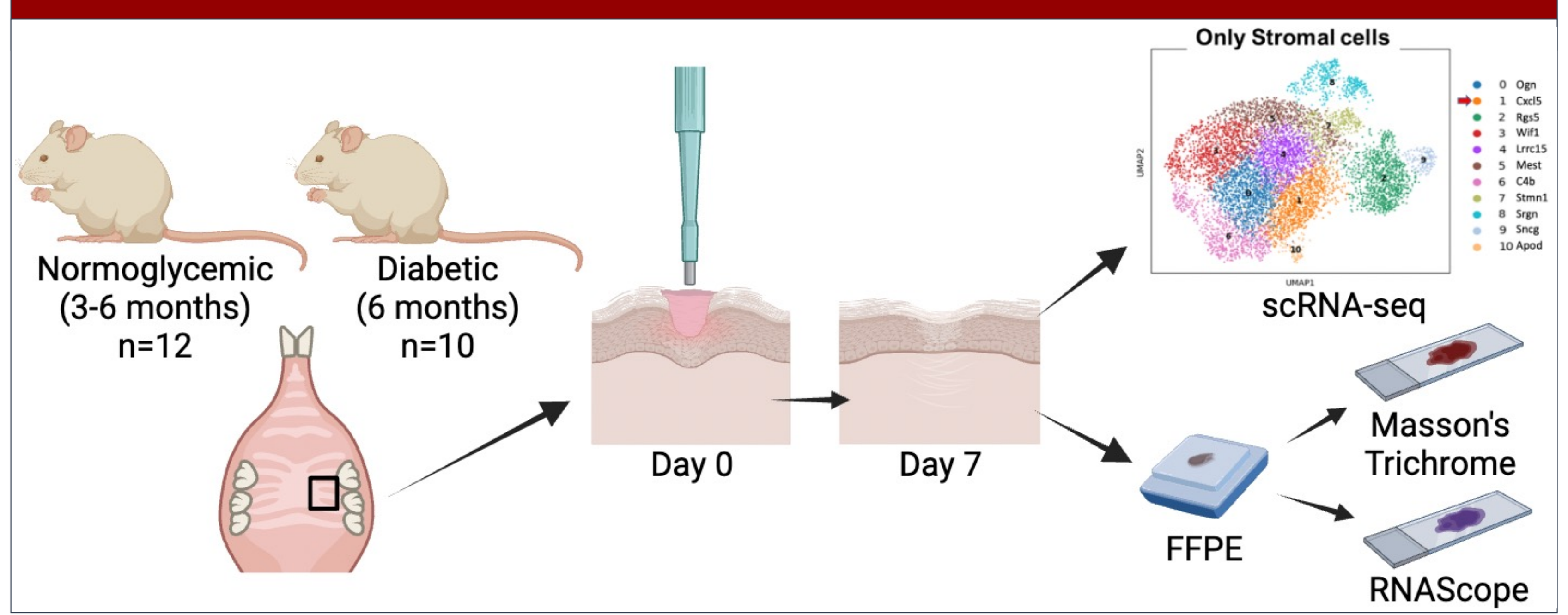
Introduction

The wound healing process is initiated when tissue is damaged. This dynamic process involves activation of various stromal cells, such as fibroblasts, in the wound site. Recently, studies have suggested that proinflammatory fibroblasts play a critical role in releasing cytokines that recruit immune cells to injury sites and chemokines like CXCL12.[3] Systemic conditions like diabetes mellitus can impair the wound healing process.[4]

In this study, we aim to identify the inflammatory fibroblast phenotype in wounded oral mucosa of diabetic mice.

Materials and Methods



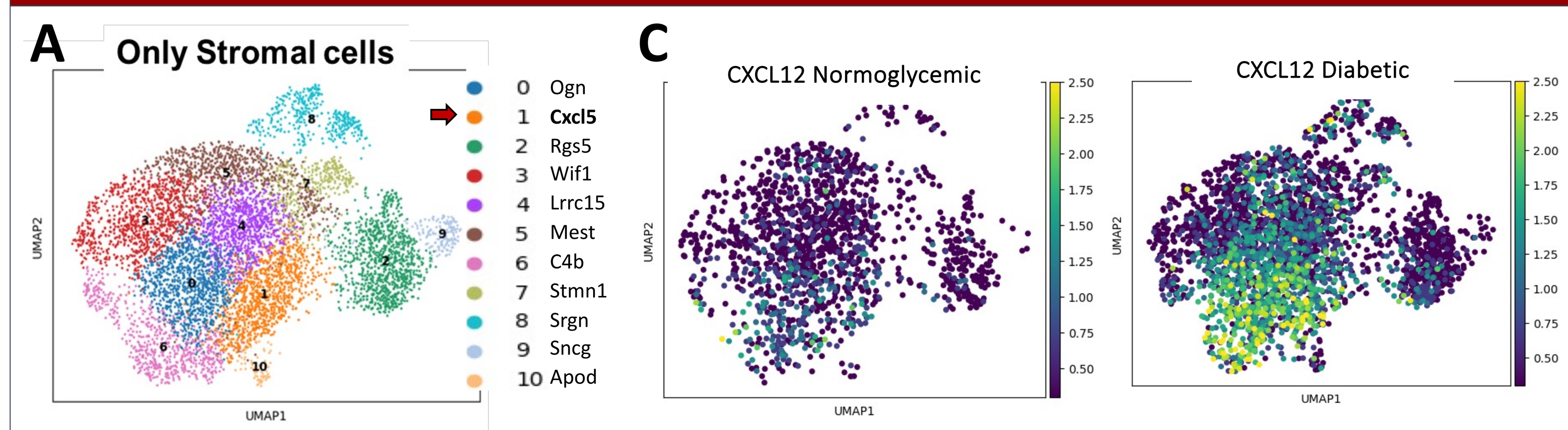
Results

There is impaired oral mucosal wound healing in diabetic mice in comparison to normoglycemic (NG) mice. Although there are no significant differences in wound gaps, there was a higher frequency of open wounds in diabetic mice.

Collagen deposition was impaired in diabetic mice with an 8% reduction compared to normoglycemic counterpart ($p<0.05$).

Additionally, scRNA-seq analysis revealed higher expression of CXCL12 in diabetic fibroblasts, which was confirmed by RNAScope analysis using the CXCL12 and PDGF receptor alpha marker ($p=0.0655$) which targets fibroblasts.

Results



B

Gene Name	Log Fold Change	% in Cluster 1	% in Other Cells	p-value
CXCL5	3.24	75.2	32.8	8.12×10^{-163}
CXCL12	2.34	79.5	48.1	2.24×10^{-140}
CCL7	2.42	71.8	38.8	5.76×10^{-107}
CCL2	2.08	75.4	43.8	2.67×10^{-92}
CXCL1	1.87	88.1	68.5	1.80×10^{-88}
PDGFRA	1.33	83.2	60.4	6.88×10^{-83}

Figure 1. Single-cell RNA sequencing identify inflammatory fibroblast enrichment in diabetic oral mucosal wounds.
(A) Uniform manifold approximation and projection (UMAP) of oral mucosal wounds in NG and diabetic mice. (B) Summary of top marker genes of Cluster 1. Log fold change is a comparison between Cluster 1 and all the other clusters (C) CXCL12 expression in stromal subcluster in NG and diabetic mice.

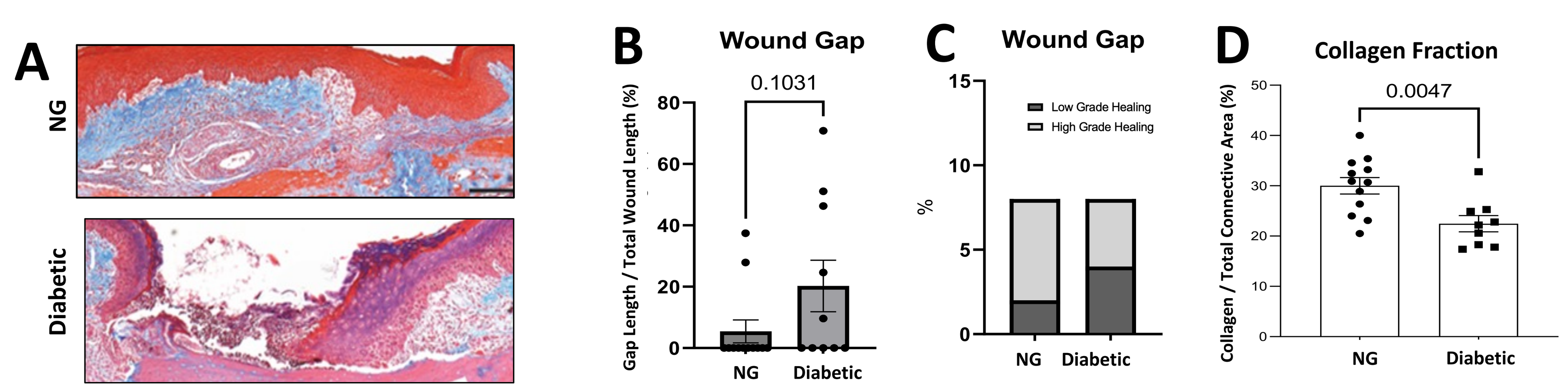


Figure 2. Decreased collagen deposition in wounded oral mucosal wounds of diabetic mice.
(A) Representative images of Masson's Trichrome staining 7 days post-wounding in NG and diabetic mice. (B) Wound gap comparison of 7 days post-wounding in normoglycemic and diabetic mice (n=10-12 per group). (C) Wound gap analysis with stratifications of low- or high-grade healing. (D) Comparison of collagen fraction ($p<0.05$) between NG and diabetic mice.

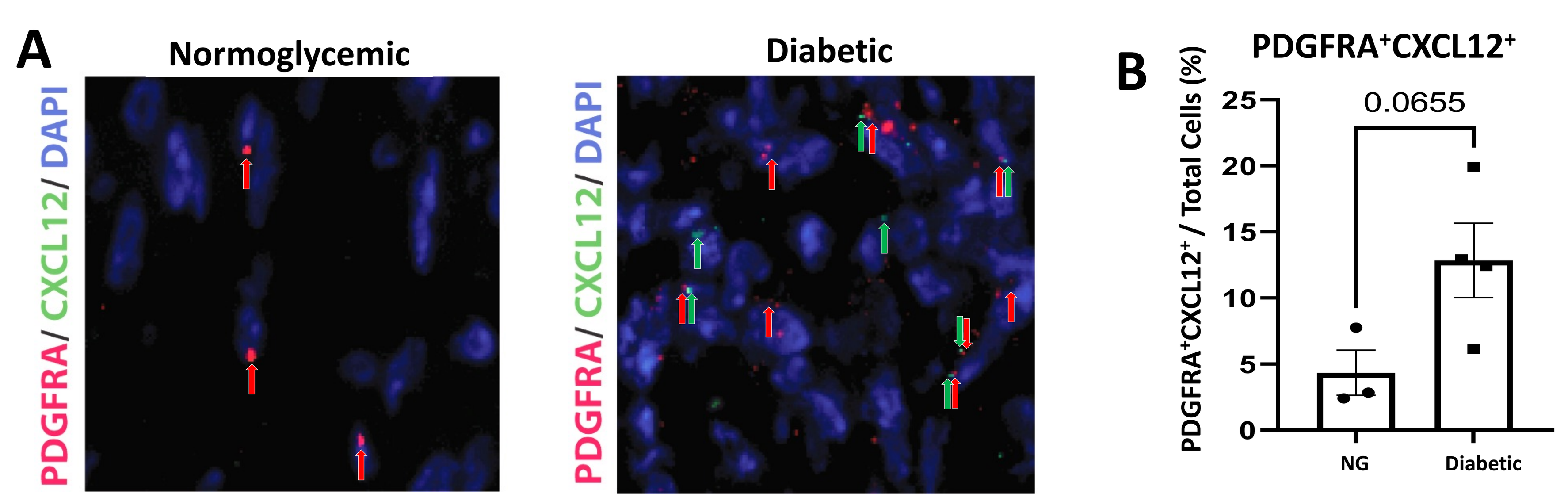
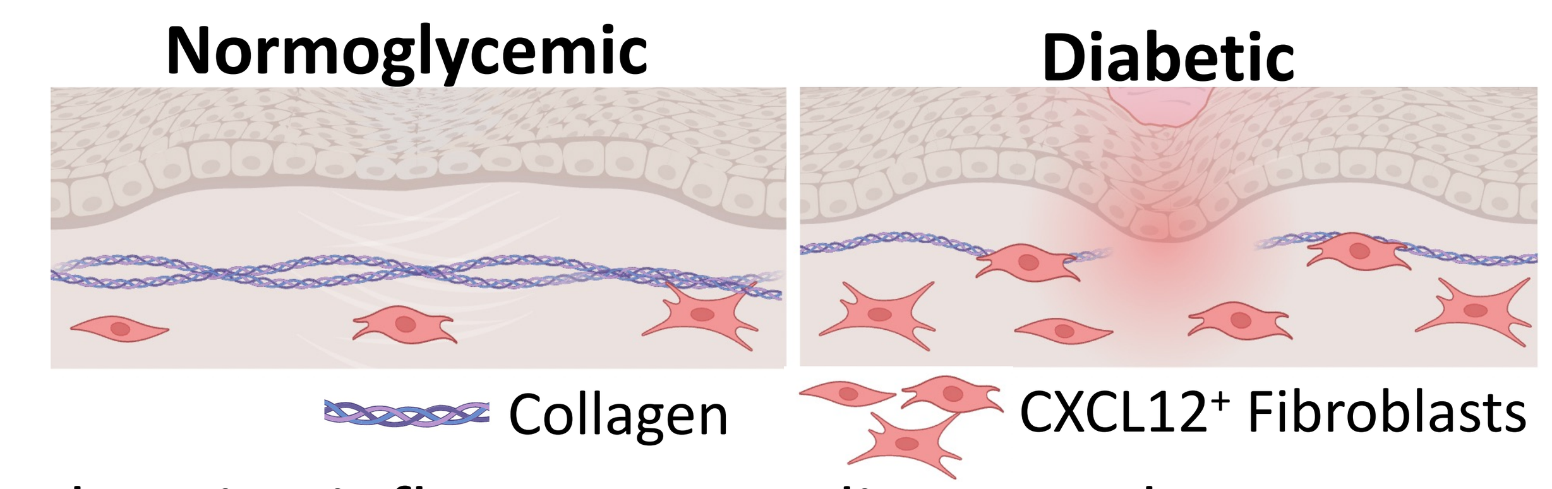


Figure 3. RNAScope shows greater amount of PDGFRA^{+} and CXCL12^{+} cells in diabetic mice.
(A) RNA in-situ hybridization images 7 days post-wounding in normoglycemic and diabetic mice stained with DAPI (blue), CXCL12 (green), and PDGFRA (red). (B) Comparing PDGFRA^{+} and CXCL12^{+} over total cells in percentage in normoglycemic and diabetic mice.

Discussion and Conclusion

Our results display an enrichment of CXCL12+ fibroblasts and reduced collagen deposition in the connective tissue of diabetic compared to normoglycemic mice.



Several chronic inflammatory diseases have reported an inflammatory fibroblast subtype in inflamed tissues.[5,6] This population subtype is enriched in the CXCL-chemokine family namely CXCL1 and CXCL12.[6] Functionally, inflammatory fibroblasts can recruit granulocytes.[7] Our results are in line with proinflammatory fibroblast phenotype description as we found an increase in CXCL5, CXCL12 and CXCL1 in diabetic mice. Future research should address mechanisms dictating CXCL12^{+} fibroblast cell fate in diabetic conditions, its impact on other cell recruitment, and shed light on treatments to rescue the diminished collagen production.

References

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