# IL33+ Fibroblasts Activate Regulatory T Cells to Facilitate Rapid Wound Healing in Oral Cavity

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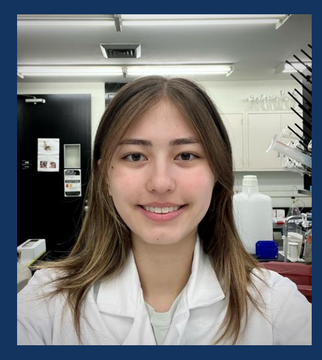

## Abstract

The role of regulatory T cells ($T_{regs}$) in autoimmunity and cancer is well-understood. However, their role in facilitation of oral wound healing requires further investigation. In this study, we aimed to understand how the ablation of FOXP3+ Tregs affects oral wound healing. Our findings suggest that $T_{regs}$ help facilitate rapid wound healing in the oral cavity by recruitment of macrophages and stimulation of their polarization.

## Introduction

Wound healing is a process that relies on complex cell signaling to yield prime results.[1] It is well-established that the oral cavity has heightened healing capacity in comparison to cutaneous tissue injury.[2] Previous research in our lab has shown that interleukin-33 (IL-33), an alarmin cytokine that is produced mostly by fibroblasts in the oral cavity, plays a key part in oral wound healing. Injury leads to the release of IL-33 and activates Tregs that express ST2, a receptor for IL-33. We hypothesized that ablation of FOXP3$^+$ Tregs will negatively impact healing in the oral cavity. Treg deletion was achieved by diphtheria toxin injection into mice genetically modified to express diphtheria toxin receptor specifically in FOXP3$^+$ Tregs. Wounded palatal tissue from mice with Treg deletion and control mice was collected after 2 and 4 days post injury for histomorphometric analysis. Mice that had IL33 deleted in Col1a2$^+$ fibroblasts were also examined via immunofluorescence by CD206 and F4/80 double stain to detect pro-resolving macrophages.

## Results

Macrophages that resolve inflammation, identified by CD206$^+$ and F4/80$^+$ immunopositivity, were reduced in the oral wounds of experimental mice that had IL33 deleted compared to control groups in both day 2 and 4 wound specimens. In mice that had Tregs ablated, there was significantly delayed wound healing, evident by slower wound gap closure (wider epithelial gap) when compared to control groups in day 2 wounds. However, in the scalp samples (Figure 1), there was no significant difference in healing which eludes to fibroblast-derived IL-33 having a significant impact on the healing of oral wounds rather than cutaneous ones.

## Discussion

Our results suggest that IL33-ST2 axis via stromal-Treg interaction plays a significant role in the facilitation of oral wound healing. Combined with our previous findings, this supports an important role of fibroblast-leukocyte crosstalk to quickly trigger resolution of inflammation pathway, which is necessary for rapid regenerative healing. Further investigation is warranted to identify how activation of Tregs via IL33-ST2 shifts macrophage polarization from pro-inflammatory to pro-resolving.

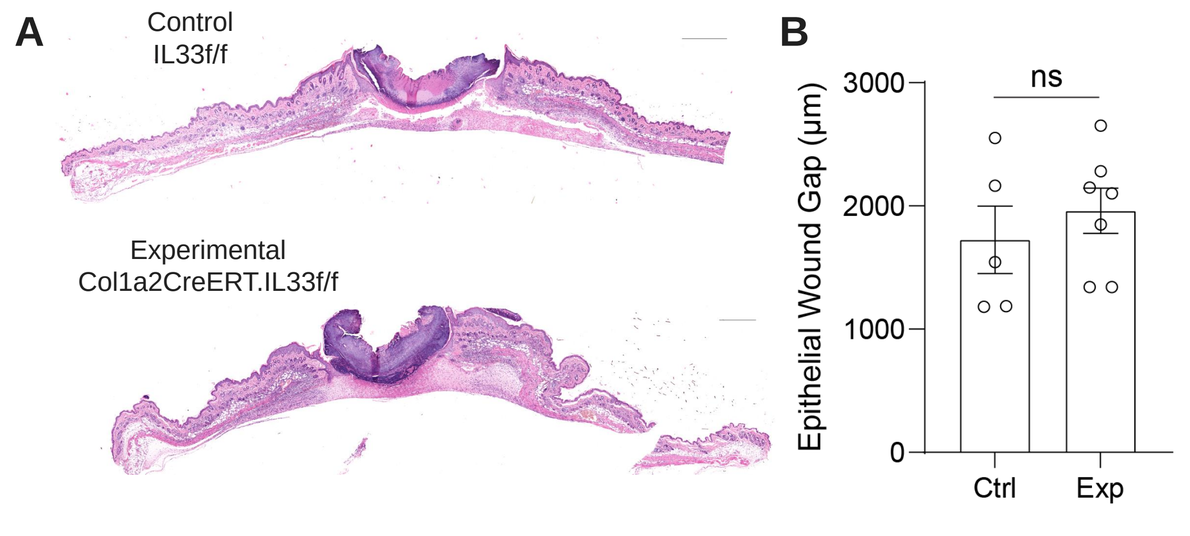


**Figure 1. (A)** Representative images demonstrate similar scalp wound gap closure on day 4 after injury between control and experimental group. Scale bar, 500 µm. **(B)** Quantification of epithelial gap closure. Each image represents mouse scalp wound sample. n=5 samples; stained in several independent batches. Student t test, p = .1548.

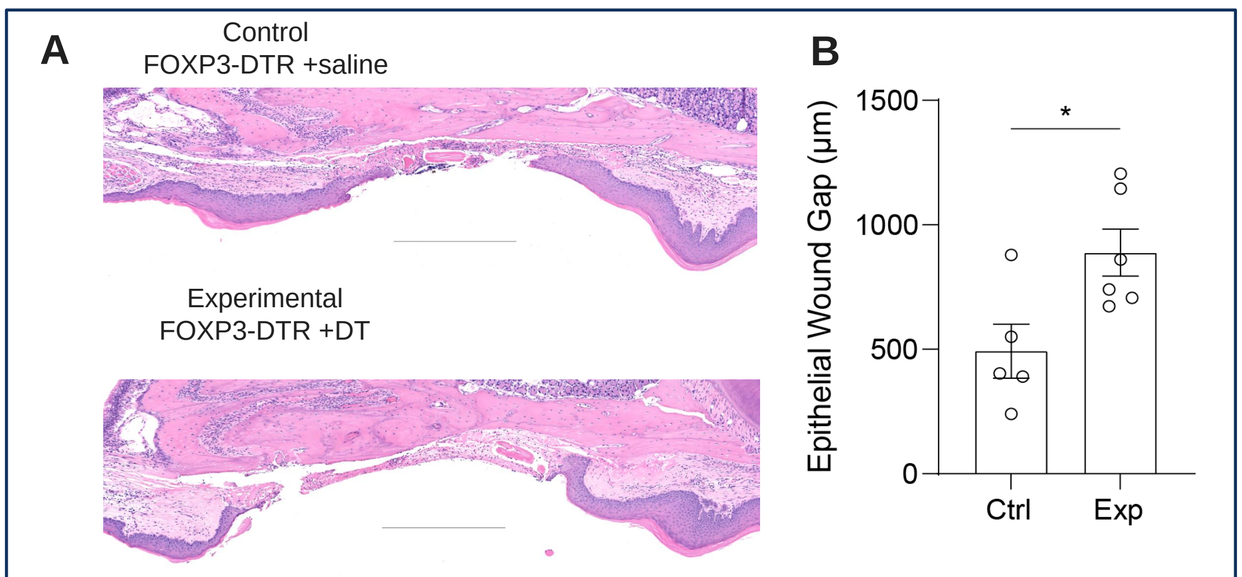


**Figure 2. (A)** Representative images indicate impaired healing in palatal experimental group. Scale bar, 500 µm. **(B)** Quantification of epithelial gap closure. Each image represents one mouse palatal wound sample. n = 5-6 samples; stained in several independent batches. Student t test, p = .022.

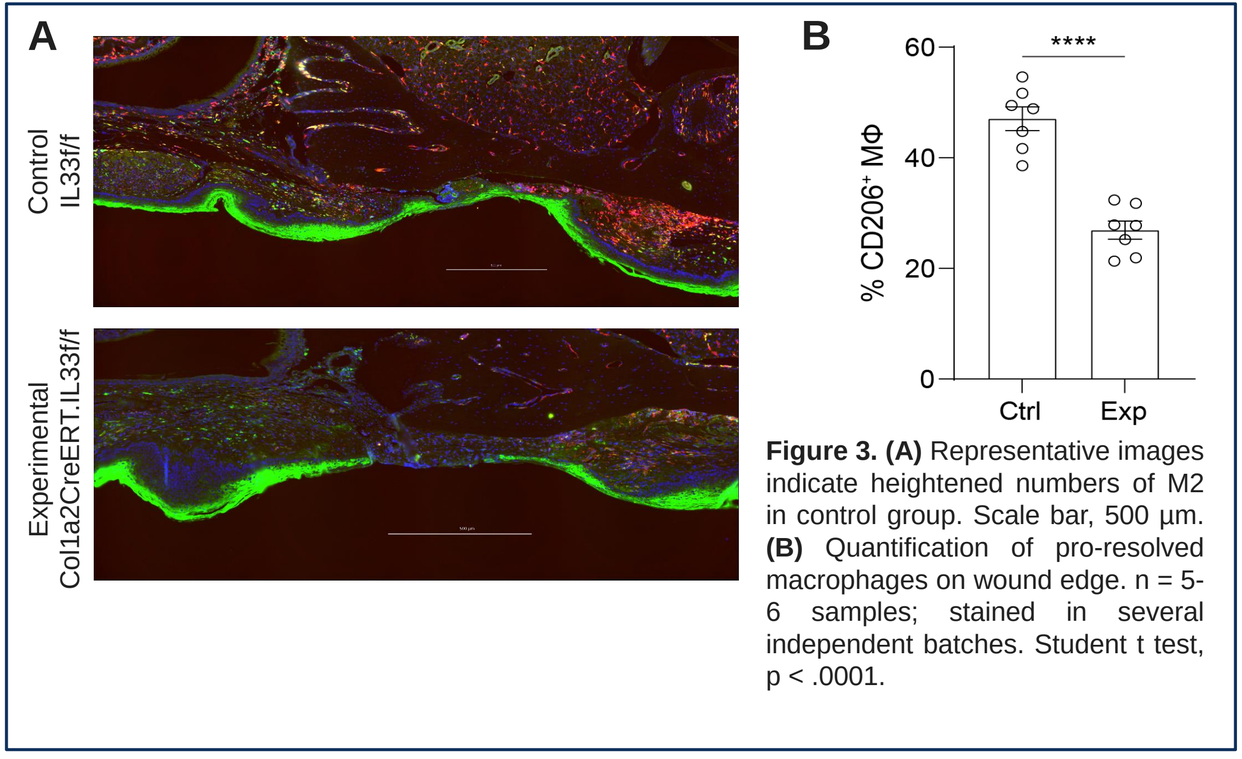


**Figure 3. (A)** Representative images indicate heightened numbers of M2 in control group. Scale bar, 500 µm. **(B)** Quantification of pro-resolved macrophages on wound edge. n = 5-6 samples; stained in several independent batches. Student t test, p < .0001.

## Conclusions

IL33 deletion in fibroblasts delays wound healing and prevents fast resolution of inflammation. Tregs, a major ST2+ expressing cell type, is necessary for rapid oral wound healing, without which leads to abnormal healing similar to that observed in IL33-deleted group. These results point to an important role of IL33-ST2 signaling in faster wound healing, which may be utilized as a therapeutic target in wounds that heal sub-optimally.

## References

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2. Toma, et al. “Oral wound healing models and emerging regenerative therapies.” Translational Research, vol. 236, pp. 17-34. October 2021. https://doi.org/10.1016/j.trsl.2021.06.003

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