

4-Aminopyridine (4AP) To Promote Wound Healing in a Type 2 Diabetic Murine Model

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Background

Diabetic wounds represent a major clinical challenge. Impaired healing is multifactorial:

- Decreased angiogenesis
- Delayed re-epithelialization
- Persistent inflammation

4-Aminopyridine (4AP) is a potassium channel blocker approved for various neuro-degenerative indications. Seminal paper by Elfar et al. in wound healing shows that 4AP enhances:

- Axonal sprouting
- Collagen deposition
- Tissue regeneration

Hypothesis

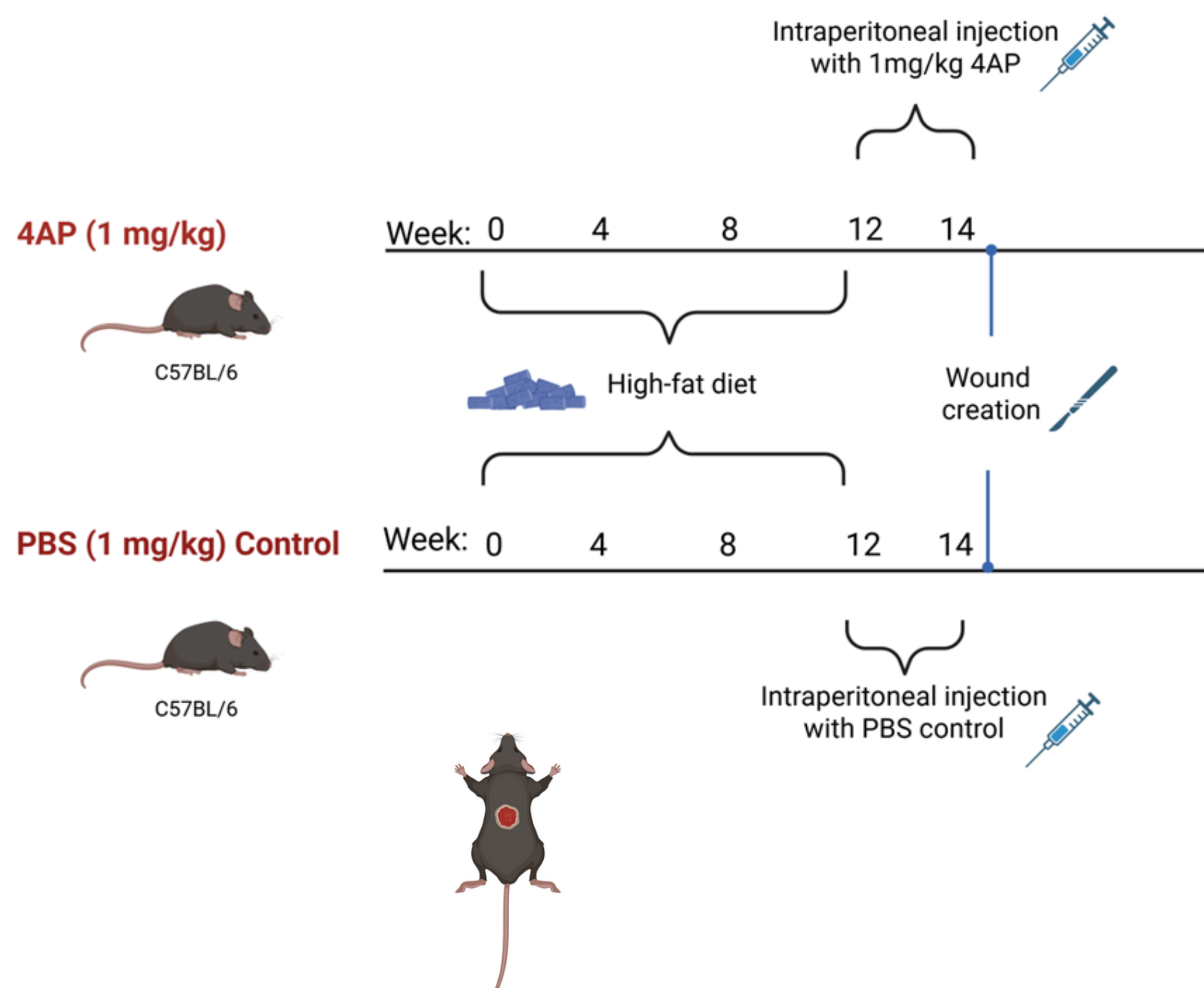
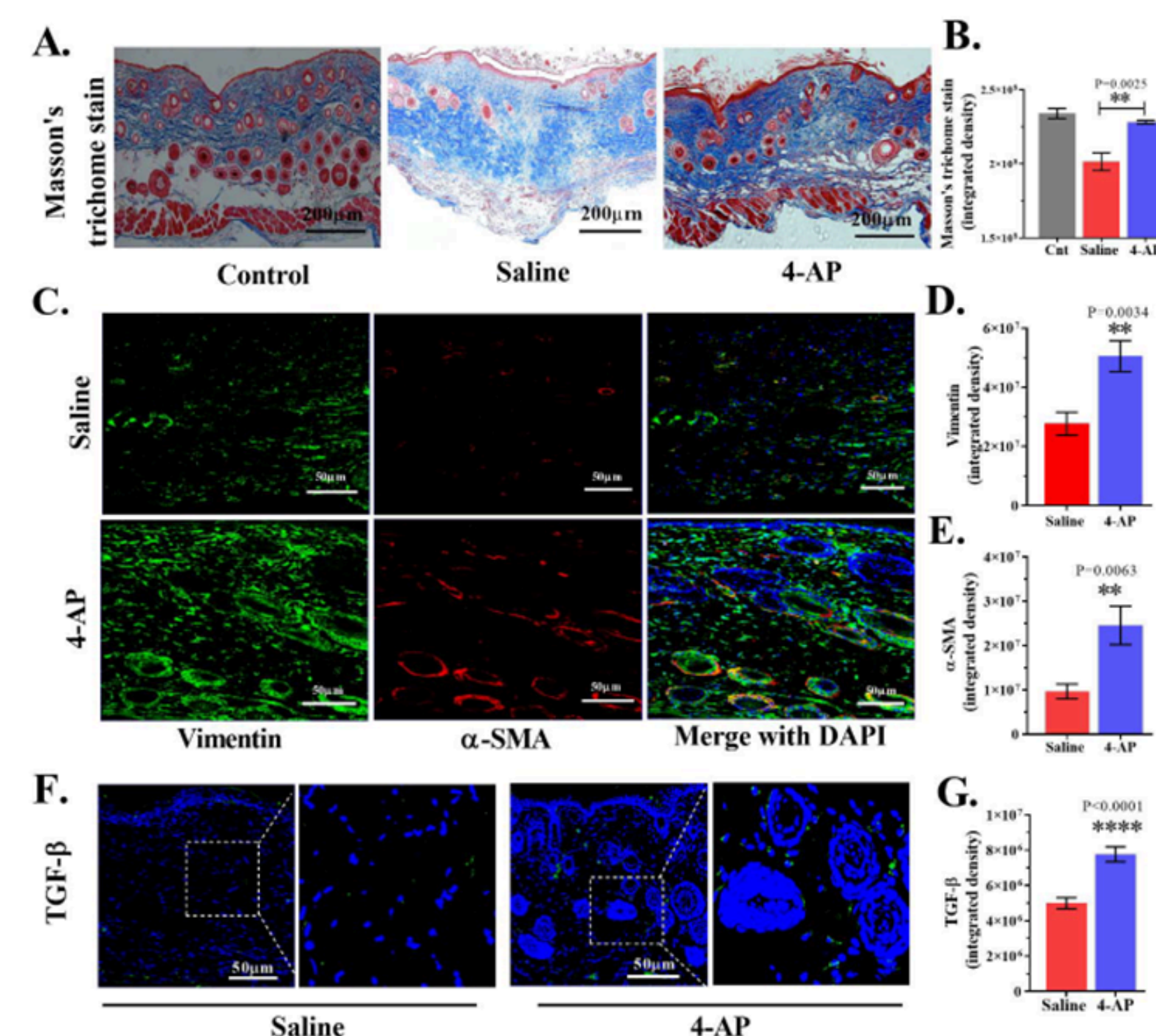
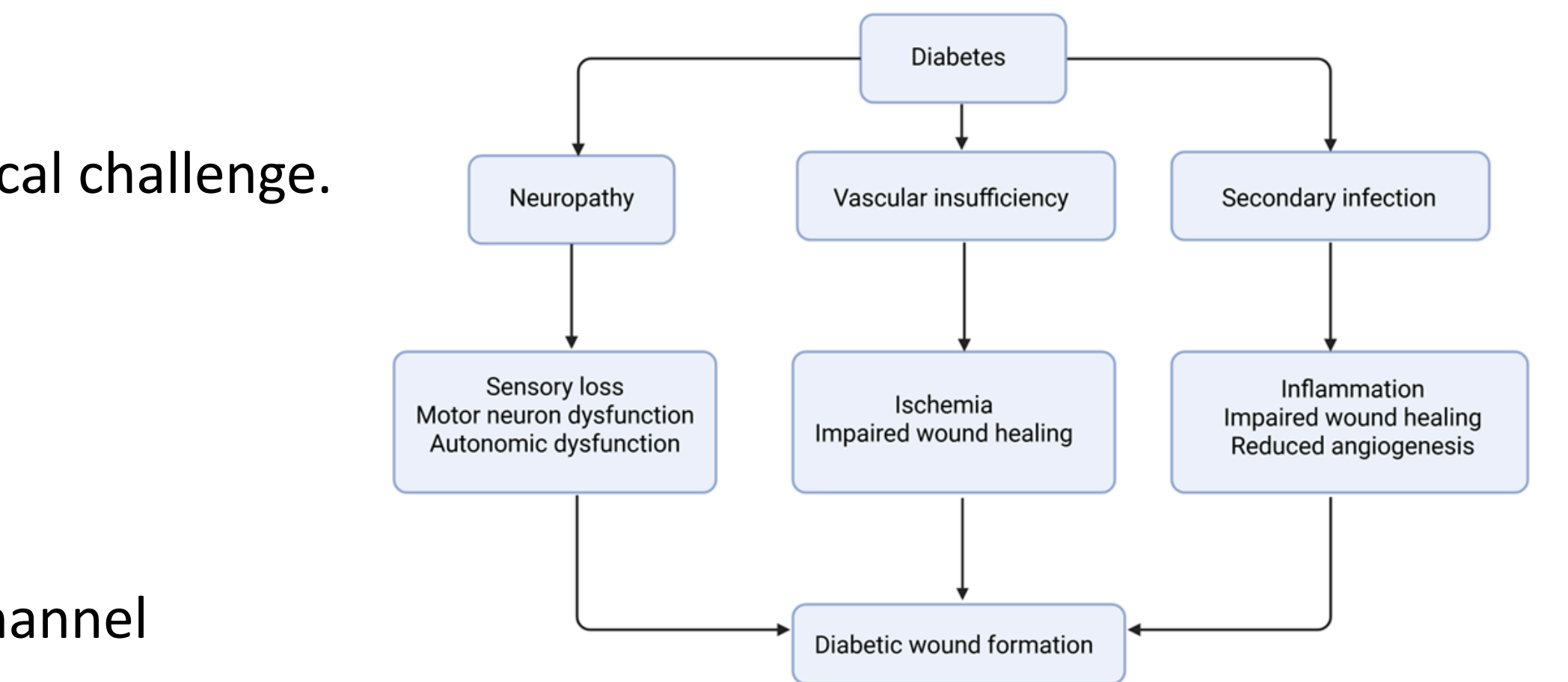
We hypothesize that 4AP promotes wound healing in diabetic wounds via pro-regenerative and anti-inflammatory effects.

Methods

- Model: C57BL/6J mice
- High-fat diet (60% kcal fat) for 12 weeks → Type II diabetic phenotype
- Intervention:
 - Week 12–14:
 - Group 1: HFD + PBS (control)
 - Group 2: HFD + 4AP (1 mg/kg)

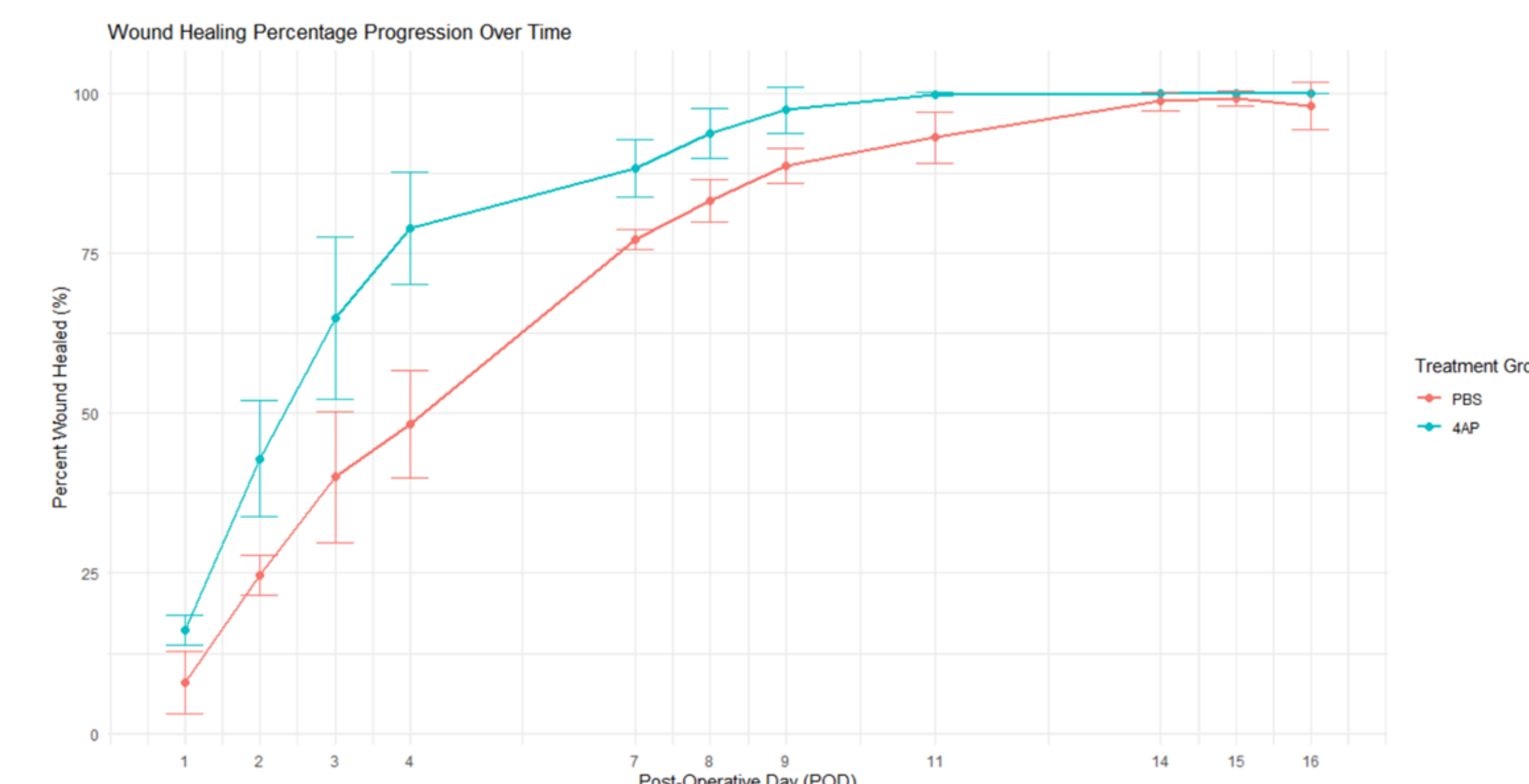
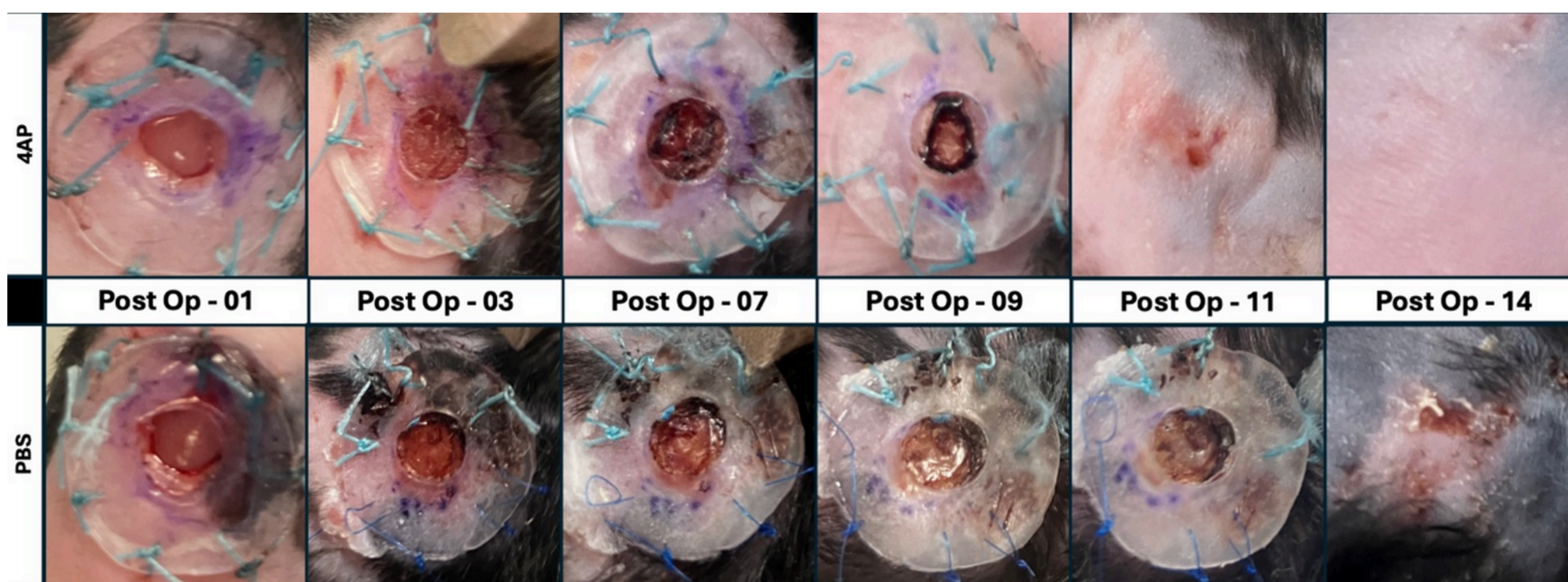
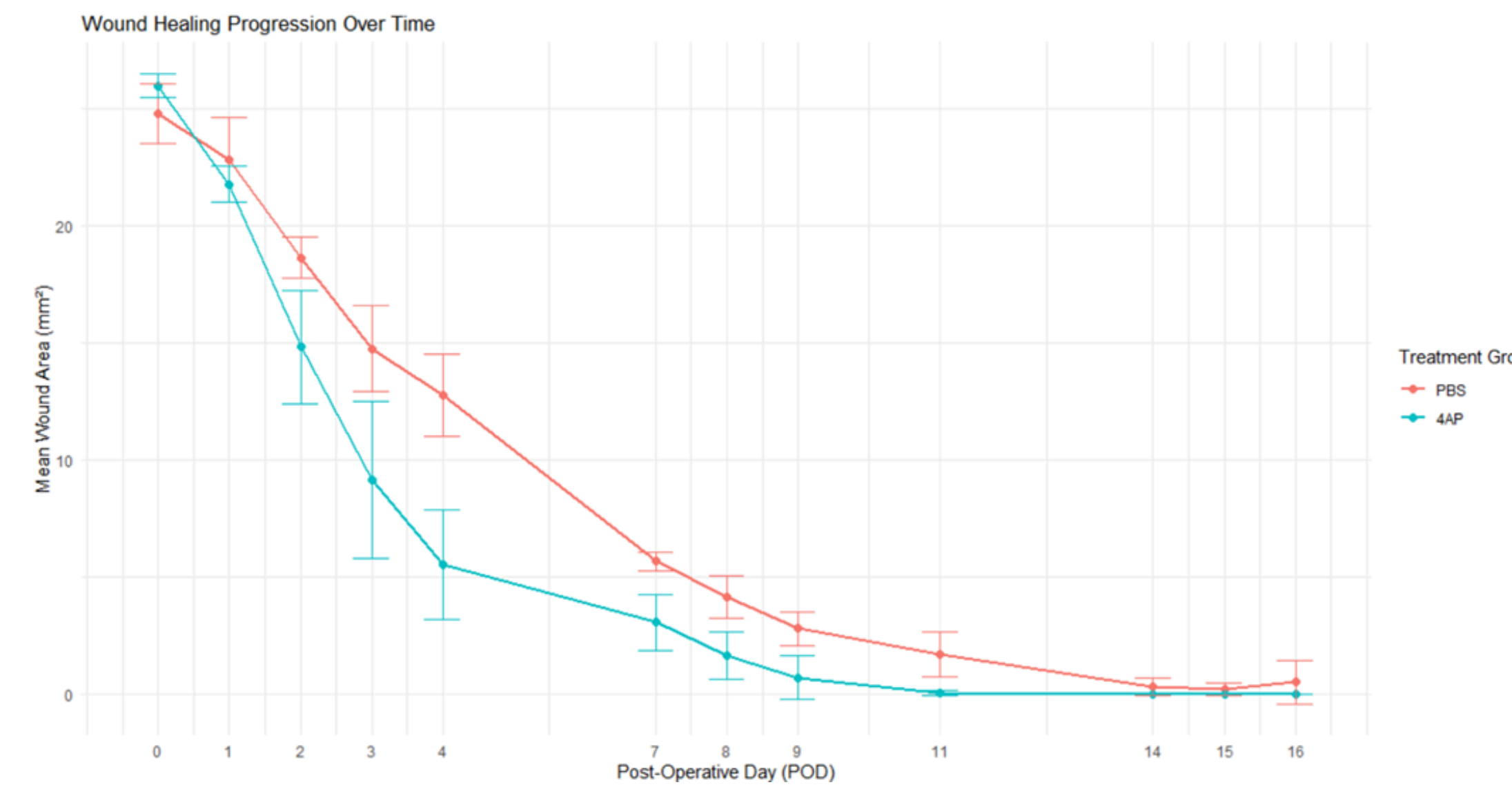
- After 14 weeks, full-thickness dorsal wounds were created, and wound healing was monitored until POD 16

- Wound Creation:
 - POD 0: 6-mm full-thickness dorsal wounds + silicone splint
 - Daily imaging through POD 16



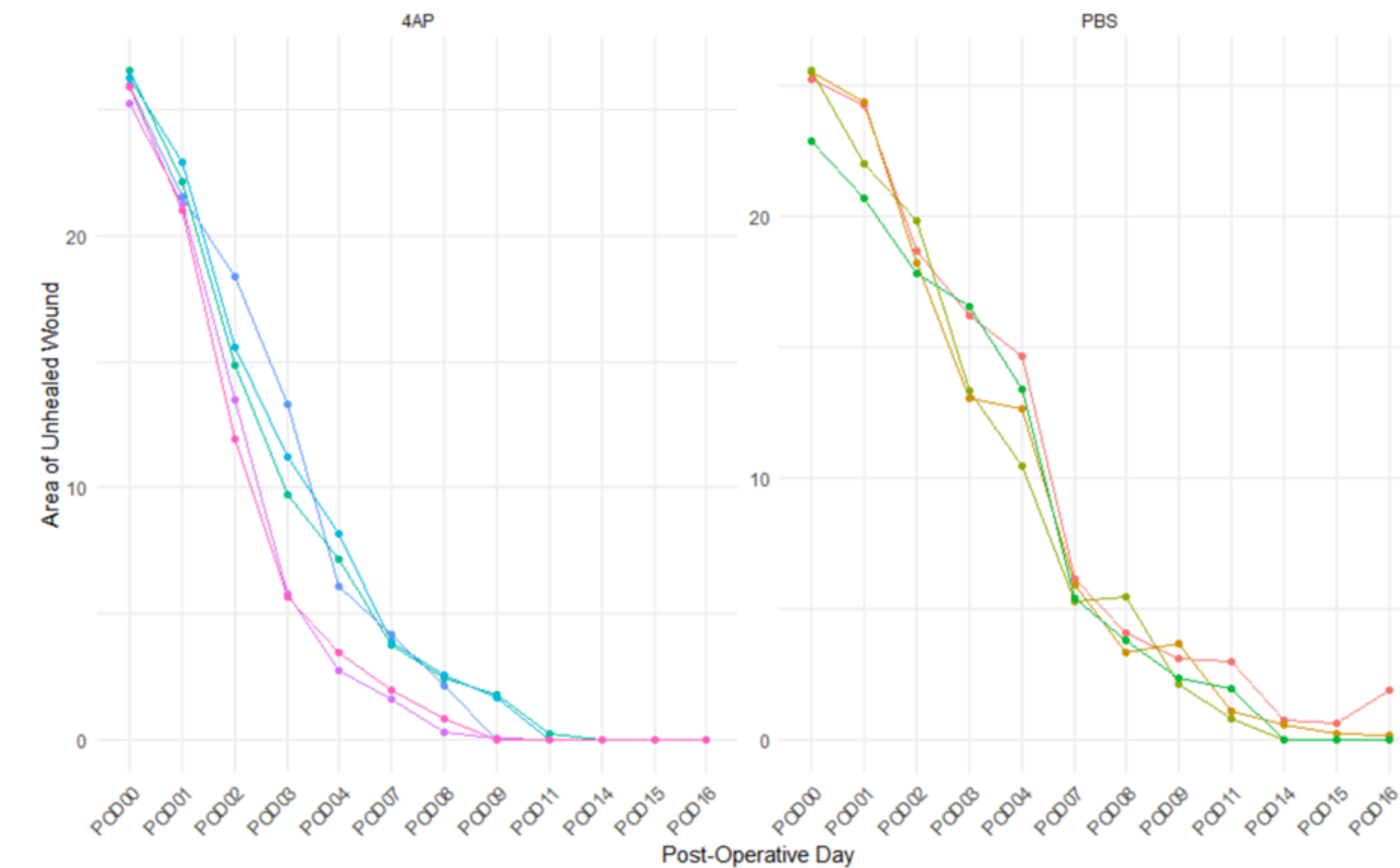
Results

- Wounds in the 4AP group contracted significantly faster from POD 2–11
- POD 4: Wound area 7.24 mm² smaller in 4AP vs. PBS (p = 0.001)
- By POD 11, wounds were nearly closed in 4AP mice (~98%)
- PBS wounds remained partially open through POD 16



- POD 2: 4AP group had significantly greater closure (p = 0.02)
- POD 7: 85% closure in 4AP vs. 70% in PBS (p = 0.005)
- POD 11: 4AP wounds >99% closed vs. 93% in PBS (p = 0.04)
- Complete closure achieved by POD 16 in all 4AP mice

Unhealed Wound Area Over Post-Operative Days by Mouse and Group



- 4AP-treated mice demonstrated uniform closure
- PBS group showed wider variability; multiple mice had incomplete healing at POD 16



Measureme nt	High-Fat PBS (Mean ± SD)	High-Fat 4AP (Mean ± SD)	Low-Fat PBS (Mean ± SD)	Low-Fat 4AP (Mean ± SD)	p-value (Group Effect: PBS vs. 4AP)	p-value (Diet Effect: HF vs. LF)	p-value (Interaction)
Epidermal Length (µm)	2097 ± 510	2808 ± 462	2764 ± 689	3479 ± 1051	0.0612	0.0789	0.9961
Epidermal Area (µm²)	132,169 ± 51,072	148,549 ± 23,619	160,155 ± 22,930	203,545 ± 90,264	0.256	0.142	0.62
Wound Length (µm)	3108 ± 460	3226 ± 590	2586 ± 570	3454 ± 670	0.635	0.414	0.339
Wound Area (µm²)	1,754,868 ± 450,000	2,878,303 ± 320,000	815,672 ± 210,000	2,583,461 ± 520,000	0.802	0.514	0.715

- Epidermal length longer in 4AP-treated mice (p = 0.0612 trend)
- Most pronounced in low-fat + 4AP group: 3479 ± 1051 µm
- Wound length and area were not significantly different, consistent with full closure in both groups

Conclusions

- 4AP significantly accelerates wound healing in diabetic mice
- Promotes early closure, epidermal regeneration, and healing uniformity
- Effect is most pronounced early (POD 2–11), with near-complete closure by POD 16
- Histomorphometric trends support enhanced epithelialization
- Translational implication: Oral 4AP may offer a low-risk adjunct for chronic diabetic wounds

Future Directions

- Mechanistic studies: Inflammatory markers, fibroblast activity, keratinocyte migration
- Longitudinal tracking of nerve regrowth + vascular remodeling
- Combination therapies: 4AP + biologics, scaffolds
- Clinical translation: Leverage 4AP's existing FDA approval for repurposing

References

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2. Galiano RD, Michaels J 5th, Dobrynsky M, Levine JP, Gurtner GC. Quantitative and reproducible murine model of excisional wound healing. Wound Repair Regen. 2004 Jul-Aug;12(4):485-92. doi: 10.1111/j.1067-1927.2004.12404.x. PMID: 15260814.