

Cell-Free Human Amniotic Fluid (cfAF) in the Treatment of Chronic Venous Leg Ulcer in a Patient with Type 2 Diabetes and Chronic Venous Insufficiency: A Case Study

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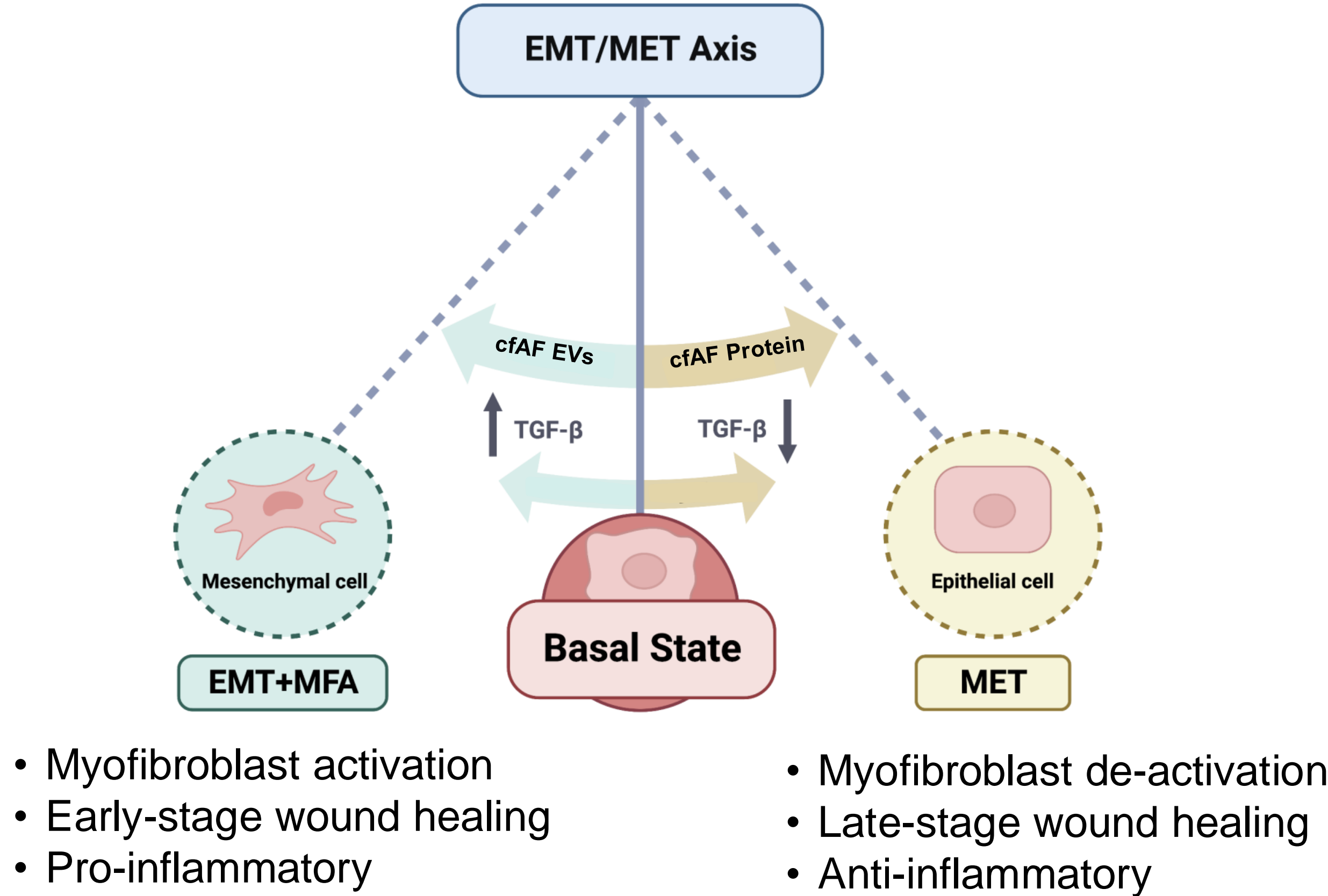
Introduction & Background

- In patients with chronic venous disorders (CVD), coexisting diabetes increases the clinical complexity of care¹.
- Venous leg ulcers (VLU) are difficult to heal with only **50% of these ulcers resolving within 12 months**².
- Currently, there are no regenerative medicine drugs approved by US FDA to promote tissue repair of VLUs.
- **This case report describes *cfAF treatment for a VLU that persisted for 37 months** in a type 2 diabetes patient that failed multiple standard of care wound interventions including debridement, compression therapy, and various skin substitutes.

Clinical Rationale & Mechanism

- cfAF extracellular vesicles (EVs) stimulate myofibroblast activation (MFA) and epithelial to mesenchymal transition (EMT)³.
- Successful early-stage healing events include MFA and EMT.
- Soluble proteins isolated from cfAF contribute to resolving EMT/MFA via mesenchymal to epithelial transition (MET)³.
- Successful late-stage wound healing events include MET mediated by cfAF repression of TGFβ-activated EMT and MFA⁴.

Figure 1. cfAF Cellular Process Modulation



Case Description

- A 73-year-old African American male with a history of CVD, type 2 diabetes (HbA1c 6.7%), hypertension, hyperlipemia, and obesity (BMI 29.3) presented with a 4.92 cm² VLU of the right lower extremity perimalleolar area.
- The VLU had persisted for 37 months and failed endovenous laser ablation and skin sub therapy, prior to initiating cfAF treatments.
- Clinical assessment: Wound % surface area reduction (PAR) over time and patient reported pain score by visual analog scale (VAS).

References

(1) Mosti, G., & Caggiati, A. (2023). Improving treatment outcomes—management of coexisting comorbidities in patients with venous ulcers. In Venous Ulcers (2nd ed., Shortell & Markovic pp. 315–325). Elsevier. (2) Jarošíková R, Roztočil K, Husáková J, Dubeký M, Bám R, Wosková V, Fejfarová V. Chronic Venous Disease and Its Intersections With Diabetes Mellitus. Physiol Res. 2023 Jul 14;72(3):280-286. (3) Liu, N., Bowen, C. M., Shoja, M. M., Castro de Pereira, K. L., Dongur, L. P., Saad, A., ... & Fagg, W. S. (2022). Comparative Analysis of Co-Cultured Amniotic Cell-Conditioned Media with Cell-Free Amniotic Fluid Reveals Differential Effects on Epithelial-Mesenchymal Transition and Myofibroblast Activation. Biomedicines, 10(9), 2189. (4) Dittmars F, Ducharme SE, Lee AM, Reems JA, Fagg WS, Markovic JN. Assessing the Safety, Tolerability and Efficacy of Cell-Free Amniotic Fluid in the Treatment of Non-Healing Venous Ulcers: Initial Experience From a Prospective, Multicenter, Phase II Study. Int Wound J. 2025 Apr;22(5) Gelfand, J. M., Hoffstad, O., & Margolis, D. J. (2002). Surrogate endpoints for the treatment of venous leg ulcers. Journal of investigative dermatology, 119(6), 1420-1425.

Biologic Treatments & Timeline

- Treatment consisted of sharp debridement and **weekly injections for 3 months with 1 mL doses** of cfAF delivered by subcutaneous injection at 3, 6, 9, and 12 o'clock positions; 3 mm lateral of ulcer margin and into the center of the ulcer bed.
- Following the 12-week injection series the ulcer was cleaned weekly at the clinic and covered with amniotic tissue or collagen foam with compression until wound closure.

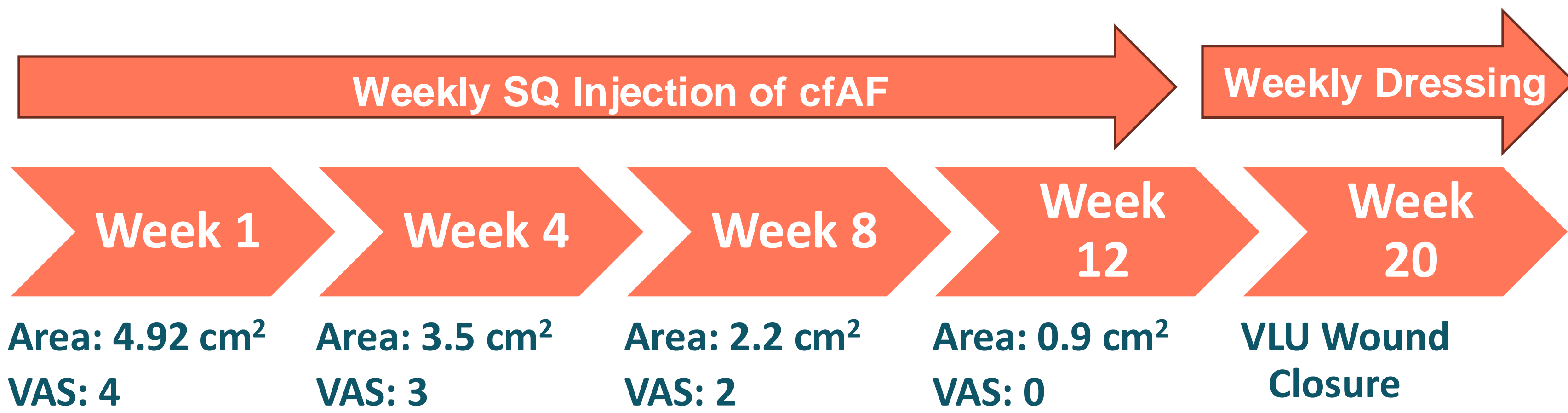


Figure 2: cfAF Application Methodology

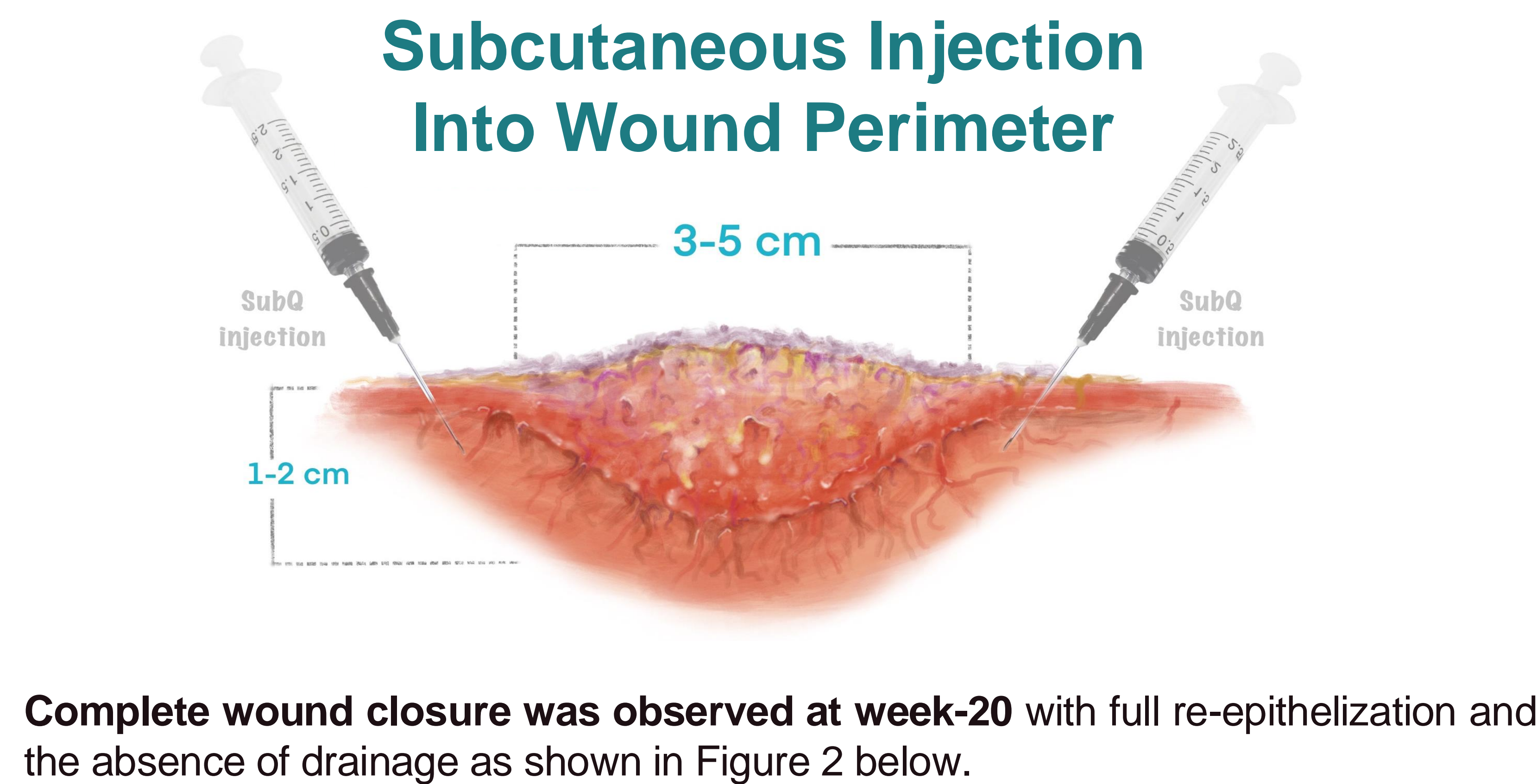


Figure 3: Complete Wound Closure at Week-20



Clinical Outcomes Measures

Figure 4. Percent Area Reduction (PAR)

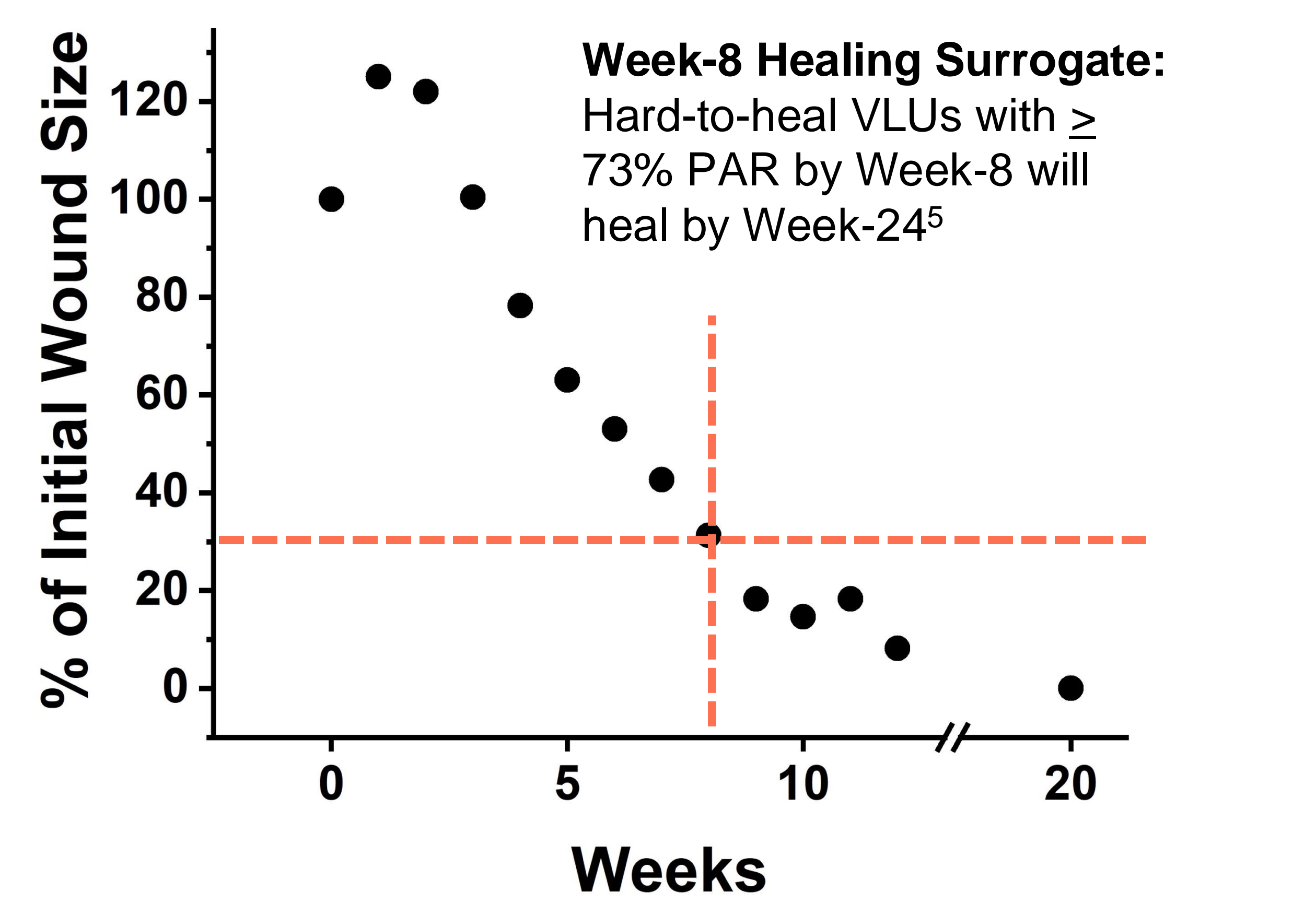
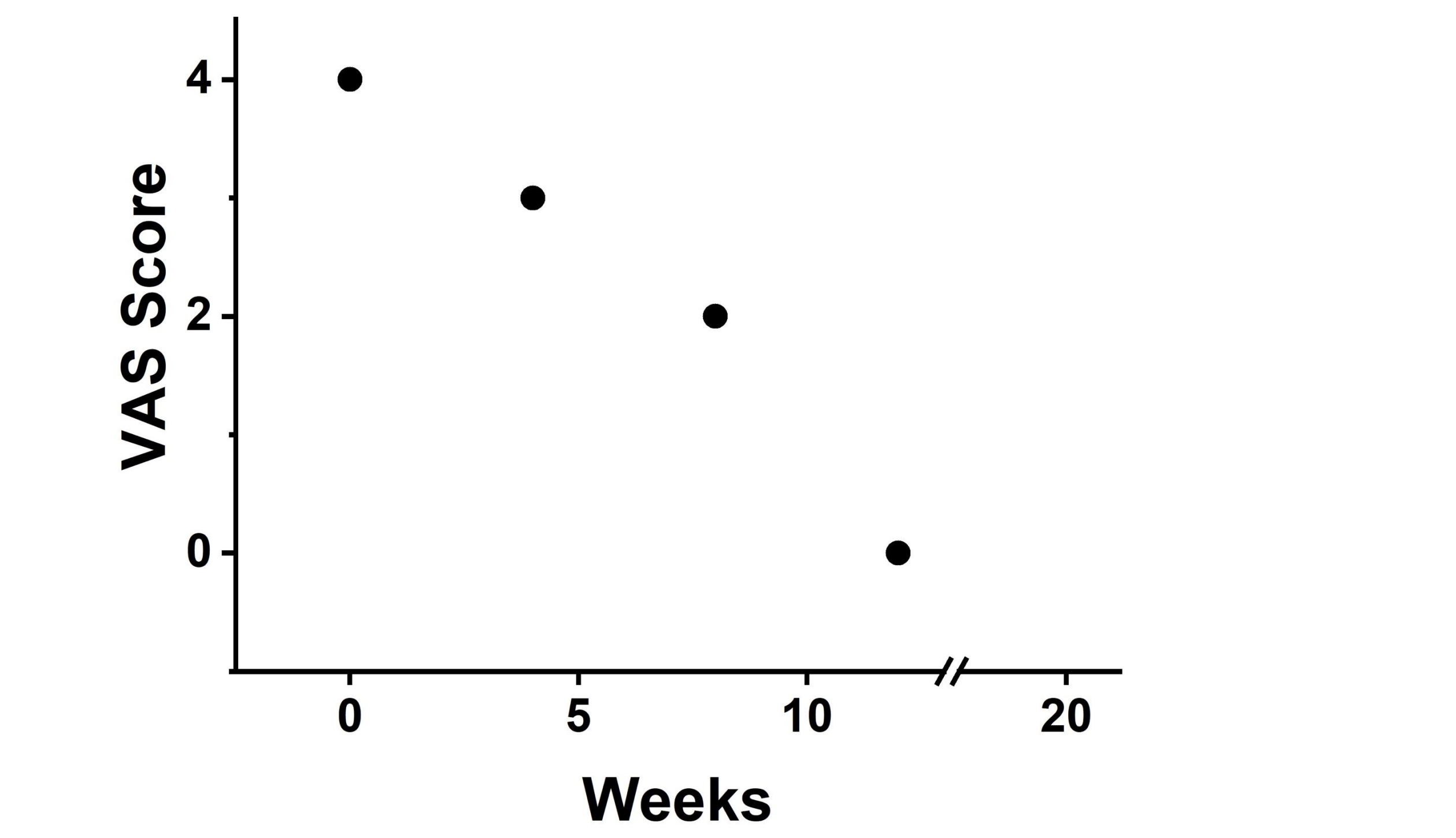


Figure 5. Visual Analog Scale



Conclusion

- VLU healed at week-20 with no ulcer pain at week-12.
- Week-8 surrogate successfully predicated healing at week-20.
- Findings presented in our study indicate that cfAF safely and effectively promoted healing of chronic VLU in a patient with multiple underlying co-morbidities.
- Larger studies are needed to determine the optimal treatment strategies for individual subsets of VLU patients.