Transcriptomic and Systemic Inflammatory Signatures Associated with Granulation Development in Combat Extremity Wounds

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INTRODUCTION

Granulation tissue formation involves coordinated inflammation, fibroblast proliferation, angiogenesis, and extracellular matrix (ECM) deposition.[1]

Transitioning from early inflammation to granulation tissue formation promotes healing of combat-related extremity wounds. Extracellular matrix production and metabolic activation are key to this process, yet wound healing is not fully understood.[1-3] We aimed to identify molecular changes associated with granulation tissue development and describe how these patterns relate to healing.

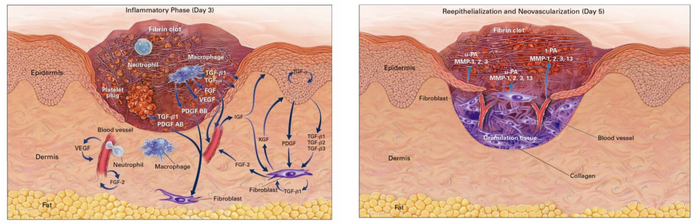
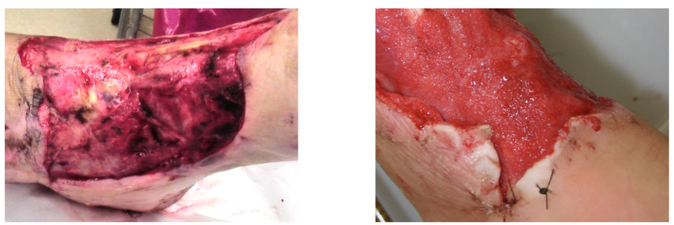


Figure 1. Adapted from Singer AJ, Clark RA. *Cutaneous wound healing.* N Engl J Med. 1999;341(10):738–746. doi:10.1056/NEJM199909023411006

METHODS

We analyzed 41 patients with 64 combat-related extremity wounds sustained during OIF/OEF. Wound images, tissue biopsies, and serum samples were collected at each surgical debridement until delayed wound closure (DWC). Trained experts assessed granulation in wound images (Figure 2). Data were analyzed by post-injury week: W1 (0-6 days), W2 (7-13 days), and W3+ (>14 days). Tissue RNA sequencing and serum protein profiling (Luminex) were performed. Differential gene expression (DESeq2), pathway enrichment (GSEA), and tissue-serum relationships (Kendall correlation) were analyzed.



No visual signs of granulation tissue **Visual signs of major granulation tissue**

Figure 2. Combat extremity wounds demonstrating absent vs. major granulation tissue formation in patients enrolled in Surgical Critical Care Initiative (SC2i) studies.

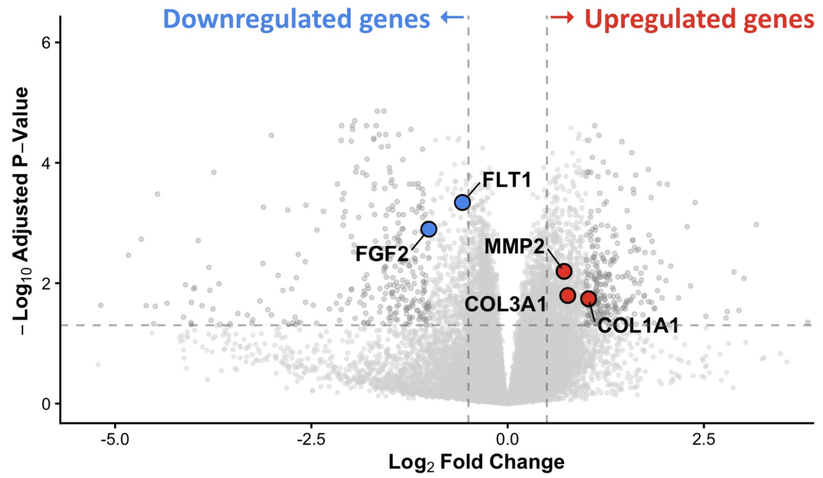


Figure 3. Expression of angiogenesis, granulation tissue formation, and ECM remodeling genes across all wound types. Analysis displays $\log_2$ fold change versus $-\log_{10}$ adjusted p-value (Benjamini–Hochberg correction for multiple comparisons), with significantly differentially expressed genes highlighted. COL1A1=collagen type I alpha 1 chain (ECM deposition). COL3A1=collagen type III alpha 1 chain (ECM deposition). MMP2=matrix metalloproteinase 2 (ECM remodeling). FLT1=fms-related receptor tyrosine kinase 1 (angiogenesis regulation). FGF2=fibroblast growth factor 2 (pro-angiogenic signaling).

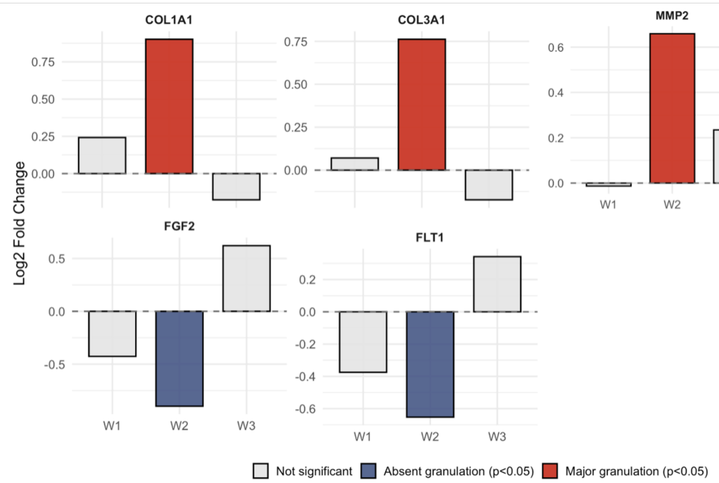


Figure 4. Temporal expression of RNA transcripts of key ECM and angiogenesis-related genes across wound healing weeks (W1–W3). Bar plots show $\log_2$ fold change by week (adjusted $p < 0.05$). Red indicates significant changes in wounds with major granulation; blue indicates significant changes in wounds without granulation. Week 2 marks the key divergence point, with ECM upregulation (COL1A1, COL3A1, MMP2) in granulating wounds and downregulation of angiogenesis-related genes (FGF2, FLT1) in non-granulating wounds.

RESULTS

Thirty-two patients (78%) with 46 wounds (72%) developed granulation prior to closure; 40 of these wounds (87%) achieved successful DWC at 30 days. Granulating wounds demonstrated upregulation of ECM genes (COL1A1, COL3A1, MMP2) with relative downregulation of angiogenic signaling (FGF2) and angiogenesis-regulating activity (FLT1) (Figure 3). Pathway analysis showed enrichment of mitochondrial translation, oxidative phosphorylation, and ATP synthesis, suggesting increased energy demand. Week 2 represented the key divergence point, with increased expression of ECM genes relative to non-granulating wounds (Figure 4). ECM genes were inversely correlated with pro-inflammatory cytokines (IL-1β, TNF-α) and FGF-basic, while FGF2 gene expression was positively associated with MCP-1 and IL-10, and FLT1 inversely associated with IL-2 (Figure 5), supporting coordinated transition from inflammation to tissue repair.

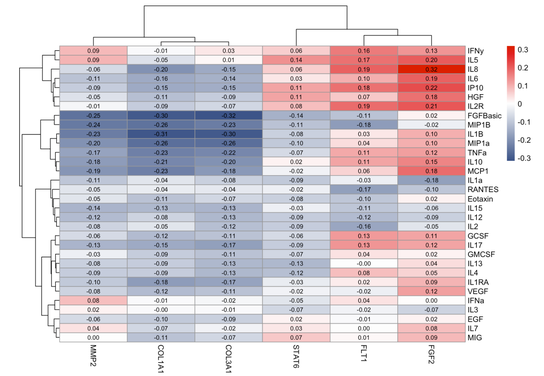


Figure 5. Correlation of granulation-associated genes to systemic signals. Kendall correlations mapped associations between systemic cytokines and signaling molecules (vertical axis) and granulation-associated gene expression (horizontal axis). Significant inverse correlations include COL1A1/COL3A1 with IL-1β, TNF-α, and FGF-basic, and FLT1 with IL-2. Significant positive correlations include FGF2 with MCP-1 and IL-10. Color scale represents correlation magnitude and direction.

CONCLUSION

Granulation tissue formation was associated with coordinated changes in extracellular matrix and metabolic gene expression, notably during week 2 post-injury. Our findings suggest a time-specific molecular pattern tracks with granulation development. Future studies should determine whether disrupting this transition impairs healing and whether these molecular features have therapeutic or prognostic value.

REFERENCES

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