Comparative Efficacy of Allograft Tissues Versus Non-Allograft Technologies in Modulating Fibrosis and Promoting Regenerative Healing

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Background: Fibrosis is a maladaptive wound-healing response that disrupts normal tissue architecture and function due to excessive ECM deposition [1], dysregulated cellular signaling, and chronic inflammation [2]. Non-allograft synthetic matrices, while structurally robust, lack the biological cues necessary to mitigate these fibrotic mechanisms. Unlike synthetic materials, allograft-derived scaffolds contribute to macrophage polarization from a pro-inflammatory (M1) to an anti-inflammatory (M2) phenotype, shifting the wound response away from fibrosis and toward functional tissue remodeling [3]. Despite the demonstrated regenerative potential of allograft tissues, comprehensive molecular and biomechanical comparisons between allograft and non-allograft materials remain limited.

Methods: A splinted excisional wound model was performed as previously described [4]. Bilateral 6-mm full-thickness wounds were created in all 48 mice. The wound beds were randomized to receive a 6-mm wound matrix consisting of either an experimental human-derived acellular dermal matrix (ADM; AlloPatch, MTF Biologics, Edison, NJ, USA) or a synthetic dermal scaffold, biodegradable temporizing matrix (BTM; PolyNovo, Carlsbad, CA, USA). Animals were allowed to recover, and full-thickness dorsal skin specimens were harvested at 7 days, 6 weeks, or 3 months post-wounding. Wounds were subsequently evaluated by bulk RNA sequencing, histologic assessment, and tensile strength testing. Bulk RNA sequencing data were further analyzed in R using principal component analysis (PCA), differential gene expression analysis with DESeq2, and gene-level comparisons based on counts per million (CPM) derived from normalized raw counts. Gene expression patterns across

comparison groups were visualized using heatmaps with z-score standardization, along with box plots depicting counts per million (CPM) for selected genes. Mechanical testing was performed using an RSA-G2 Solids Analyzer (TA Instruments, New Castle, DE, USA) and uniaxial tensile testing protocol was applied. Tensile strength across treatment conditions was evaluated with one-way repeated measures analysis of variance (ANOVA) and Tukey's post-hoc multiple comparisons test for pairwise comparisons between groups.

Results: Wounds healed at an equivalent rate in between the ADM and BTM-treated wounds (Day 7 epithelial gap: 4.02 ± 1.41 vs 4.09 ± 0.97, p=0.925; Day 7 granulation tissue: 4.06 ± 2.71 vs 2.48 ± 1.62, p= 0.225, respectively). PCA revealed clear segregation of the ADM- and BTM-treated wounds from the nonwounded and untreated skin at day 7; however, no distinct separation was observed between ADM- and BTM-treated wounds (Fig. 1). No statistically significant DEGs were identified between groups based on adjusted p-values. Inflammatory gene expression (Il1b and Tnf) was elevated in both ADM- and BTM-treated wounds compared to untreated wounds, whereas Ccl2 and Il10 expression was increased only in BTM-treated wounds and not in ADM-treated samples (Figs. 2–3). In the angiogenesis domain, all four evaluated genes were upregulated in ADM-treated wounds, while only two genes (Flt1 and Vegfa) were increased in BTM-treated wounds. Across all four wound healing domains, no statistically significant differences in gene expression were observed between ADM- and BTM-treated wounds, suggesting comparable wound healing and fibrotic responses to both matrices. Consistent with the transcriptomic findings, mechanical strength testing of week 6 harvested wounds revealed comparable strength of both the ADM- and BTM-treated wounds relative to non-wounded skin.

Conclusion: Both the experimental human-derived acellular dermal matrix and the synthetic dermal scaffold, biodegradable temporizing matrix demonstrated comparable wound healing and fibrotic outcomes relative to non-wounded skin across key domains, including inflammation,

angiogenesis, re-epithelialization, and tensile strength. The acellular dermal matrix exhibited consistent transcriptomic evidence of enhanced angiogenesis across the four selected angiogenesis-related genes, while maintaining inflammatory gene expression patterns (Ccl2, Il10) comparable to non-wounded samples. Additionally, no significant differences in healed wound mechanical strength were observed between either matrix and non-wounded skin, further supporting the human-derived matrix's ability to promote adaptive wound healing without evidence of maladaptive fibrosis. Future studies evaluating the human-derived acellular dermal matrix in clinical human populations are warranted to validate these findings and support its broader application as a wound matrix.

Keywords: N/A

Clinical Trial Registry: NA

Data Deposition: None

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Competing Interests: Dr. Robert Galiano is a consultant for MTF Biologics. All other authors declare that they have no relevant conflicts of interest.

Ethical Approval: The study was approved by the Northwestern University Institutional Animal Care and Use Committee (Protocol ID: IS00032357)

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Tables and Figures:

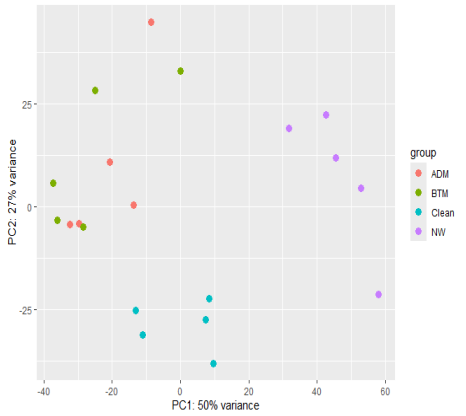


Fig. 1. Principal component analysis. PCA was performed on non-wounded skin and day 7 post-injury wounds from non-treated, ADM-treated, and BTM-treated groups.

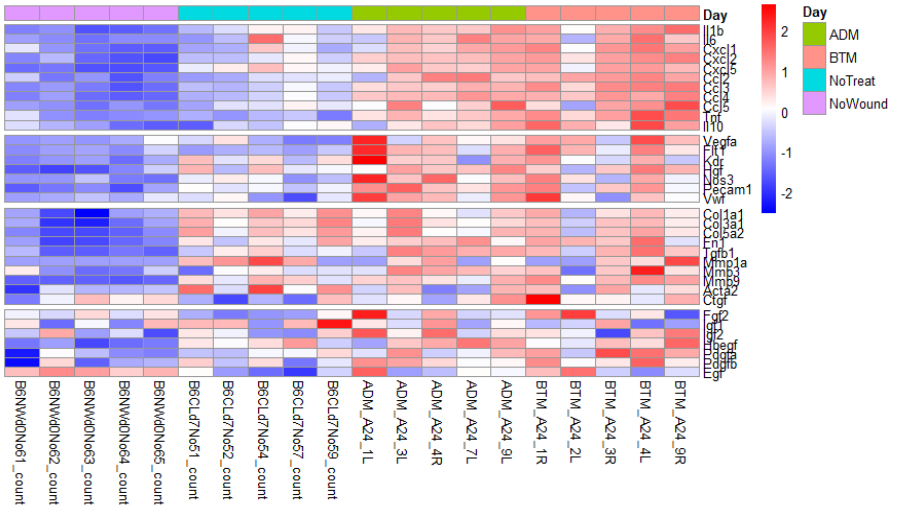


Fig. 2. Relative z-score calculated from CPM values for selected genes was calculated and visualized in a heatmap.

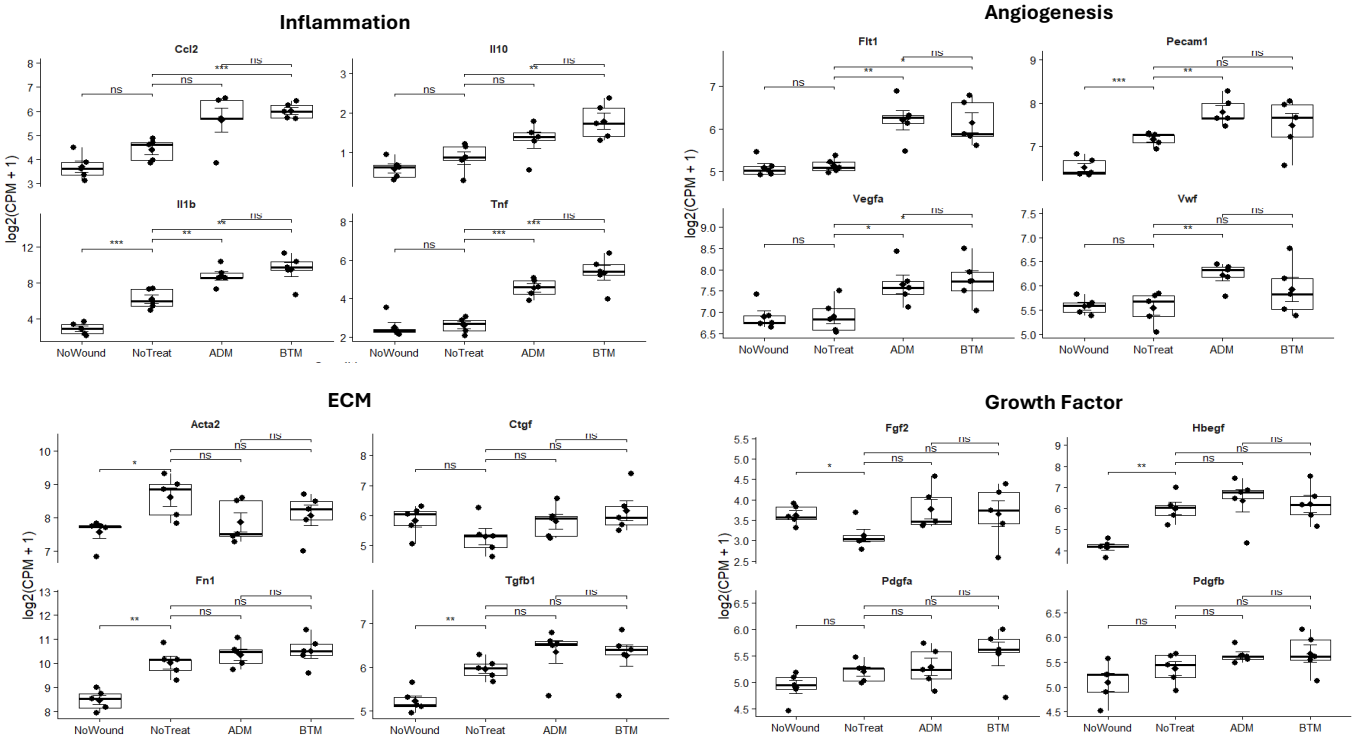


Fig. 3. CPM expression values for genes associated with inflammation, angiogenesis, extracellular matrix (ECM) remodeling, and growth factor signaling in non-wounded skin, untreated wounds, ADM-treated wounds, and BTM-treated wounds at day 7 post-injury.

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Comparative Efficacy of Allograft-Derived Tissues & ECMs Versus Non-Allograft Technologies in Modulating Fibrosis and Promoting Regenerative Wound Healing

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Background

- Fibrosis is a maladaptive wound-healing response
  - Results from a prolonged pro-inflammatory state
- Non-allograft synthetic matrices, while structurally robust, lack the biological cues necessary to mitigate fibrosis
- Allografts provide a biologically active ECM that retains endogenous cytokines, growth factors, and proteoglycans essential for modulating the wound microenvironment
  - Shifts the wound response away from fibrosis and toward functional tissue remodeling
- There is a need for allograft alternatives

Research Objectives

- To evaluate fibrosis and regenerative outcomes of allograft versus non-allograft materials in a preclinical murine (C57BL/6 mice) model.
- To perform transcriptomic profiling of allograft- and non-allograft-treated tissues.

Methods

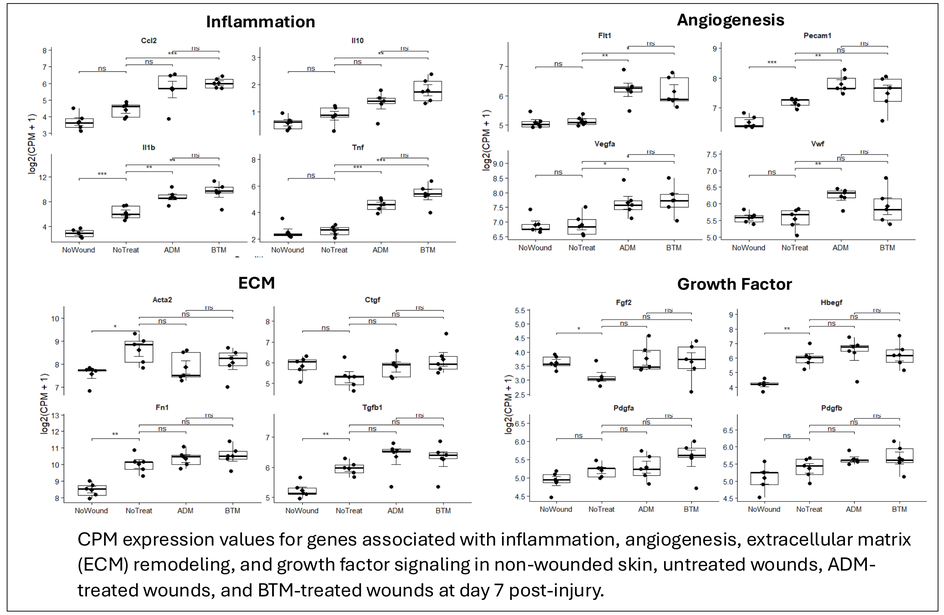
Splinted wound model with C57BL/6 mice

- Harvest at day 7, 6 weeks, and 3 months

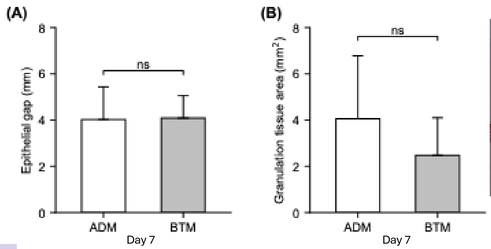
Day 7: Histology, Immunohistochemistry (IHC), cytokine analysis, bulk RNA sequencing

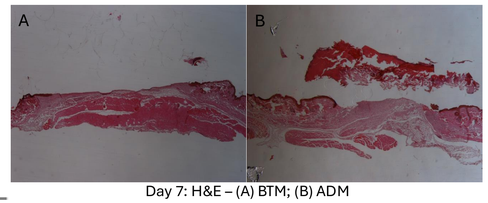
Week 6: Histology, tensile strength, protein assay, IHC

3 Month: Histology, tensile strength, persistent cytokine profiling, IHC

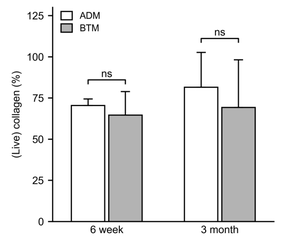


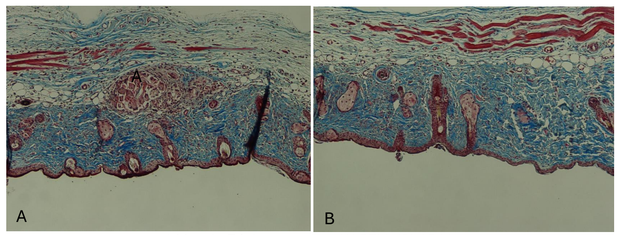
CPM expression values for genes associated with inflammation, angiogenesis, extracellular matrix (ECM) remodeling, and growth factor signaling in non-wounded skin, untreated wounds, ADM-treated wounds, and BTM-treated wounds at day 7 post-injury.





Day 7: H&E – (A) BTM; (B) ADM





3 Month: Trichrome 10x – (A) BTM; (B) ADM

Results

- ADM and BTM displayed equivocal healing at the early time point (7 days)
  - Epithelial gap: ADM 4.02 ± 1.41 vs BTM 4.09 ± 0.97; p=0.925
  - Granulation tissue: ADM 4.06 ± 2.71 vs BTM 2.48 ± 1.62; p= 0.225
- BTM with slightly more inflammation than control at day 7 on bulk RNA sequencing but no difference from ADM
- All four evaluated angiogenesis genes were upregulated in ADM-treated wounds, while only two genes (Flt1 and Vegfa) were increased in BTM-treated wounds
- Equivocal collagen maturity at later time points (6 weeks, 3 months)
- No differences in mechanical strength of healed wounds with either matrix versus non-wounded skin

Conclusion

Both the experimental human-derived acellular dermal matrix and the synthetic dermal scaffold, biodegradable temporizing matrix demonstrated comparable wound healing and fibrotic outcomes across key domains, including inflammation, angiogenesis, re-epithelialization, and tensile strength. Our findings further support the use of human-derived matrices as a viable alternative to synthetic matrices by demonstrating their ability to promote adaptive wound healing without inducing maladaptive fibrosis.