Fractional CO_2 Laser Parameter Safety in Burn Rehabilitation: Transcriptomic Thresholds from a Novel *Ex Vivo* Human Skin Model

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1. Background and rationale

Fractional CO_2 laser deposits energy as an array of microscopic columns of thermal injury, sparing intervening skin to drive rapid, orderly remodeling — the basis of **fractional photothermolysis**[1]. It is now first-line rehabilitation for hypertrophic and contracture scars, including burn scars[2], yet treatment is dosed largely by experience: energy and density are chosen empirically, and how these parameters shape the healing response at the molecular level is unknown.

Energy and density combine to give the same total fluence through very different column geometries, so the central question is **whether the outcome is set by how much energy is delivered or by how it is delivered**. We answer that transcriptionally, then ask what the high-energy program costs — whether it resolves as productive remodeling or persists toward the human scar state.

2. Methods

Model. *Ex vivo* human skin at air-liquid interface, treated with a fractional CO_2 laser at 10 or 30 mJ per pulse × 5% or 15% density, plus untreated control. *Acute study*: 24 h, 4–5 donors per arm (see legends), all five arms. *Longitudinal study*: 24 h / 3 d / 7 d, n = 3 donors, control plus the two 15% settings; RNA-seq (n = 3 per condition × timepoint) and qPCR (3 biopsies each, n = 9 per point). Independent donor cohorts.
RNA-seq & analysis. DESeq2, donor-paired; differentially expressed genes at padj < 0.05, $|\log_2 FC| > 1$. The 5%-density arms are on a separate batch, confounded with density: those contrasts are hypothesis-generating. Gene Set Enrichment Analysis (fgsea, seed 42) on Wald-ranked genes; over-representation by Enrichr (GO, Reactome). Cross-map onto published re-epithelialization[3] and hypertrophic-scar[4] signatures. qPCR ΔΔCt vs matched control (donor × timepoint), *RPLP0* reference, Wilcoxon on per-biopsy values. Benjamini–Hochberg within families.

3. Results

Same fluence, different biology

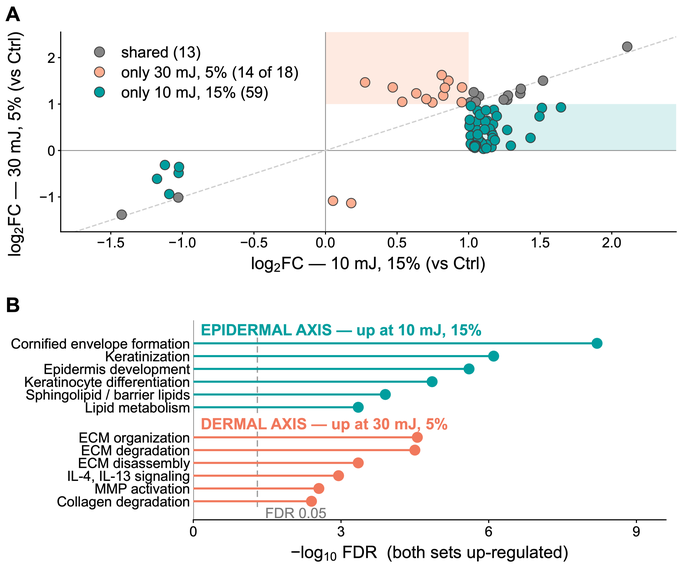


Figure 1 — At matched fluence, delivery geometry splits the response into an epidermal and a dermal program. 24 h, 10 mJ/15% (5.29 J/cm²) vs 30 mJ/5% (5.88 J/cm²), n = 4 donors. (**A**) Per-gene $\log_2 FC$ vs control. Teal, unique to 10 mJ/15% (59); coral, unique to 30 mJ/5% (18; 14 plotted, 4 off-scale); grey, shared (13). Shaded bands mark the up-regulated, setting-specific sets analyzed in (**B**) — 54 of the 59 teal and 12 of the 18 coral genes — defined as $\log_2 FC > 1$ in one setting and 0–1 in the other. Dashed line, identity. (**B**) Over-representation of the two banded sets ($-\log_{10}$ FDR; both sets are up-regulated in their own arm, so bars run in the same direction; dashed line, FDR 0.05). Teal, cornified-envelope formation (FDR 6×10^{-9}), keratinization, epidermis development, barrier lipids. Orange, ECM organization, degradation and disassembly, IL-4/IL-13 signaling, MMP activation, collagen degradation. Four low-expression artifacts (*KRT35*, *KRTAP7-1*, *MYH4*, *RPS4Y1*) excluded; the 5%-density arms are on a separate batch, so this contrast is hypothesis-generating.

Pulse energy selects the compartment — and the trade-off is measurable

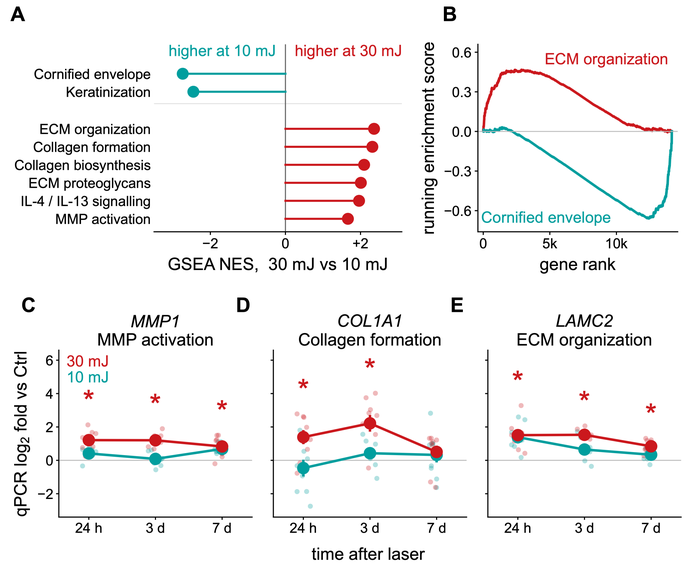


Figure 2 — At matched density, pulse energy trades epidermal differentiation against a dermal-matrix program that persists for a week. (**A**, **B**) Reactome Gene Set Enrichment Analysis (GSEA), 30 mJ vs 10 mJ at 15% density, 24 h, n = 4 donors; positive Normalized Enrichment Score (NES) = higher at 30 mJ. (**A**) Two epidermal sets down (cornified-envelope formation −2.74, keratinization −2.45), six dermal-matrix sets up (+1.66 to +2.36); all eight FDR ≤ 0.021. (**B**) Running enrichment, the two extremes; no zero crossing. (**C–E**) qPCR, n = 9 biopsies per point (8 for 10 mJ at 7 d); dots, biopsies; symbols, mean ± SEM; asterisks, FDR < 0.05 vs control at 30 mJ (per-biopsy Wilcoxon; direction concordant in 3/3 donors for MMP1 and LAMC2). (**C**) *MMP1* up at 30 mJ throughout (+1.34, +1.20, +0.83), the strongest arm discriminator (FDR 4.7×10^{-4}). (**D**) *COL1A1* peaks at 3 d (+2.69, FDR 0.026), baseline by 7 d; 10 mJ never rises. (**E**) *LAMC2* up throughout (FDR 0.010), separating the arms (FDR 0.027). *COL3A1* unchanged (FDR 0.13). (**A, B**) compare the arms with each other: against control both sit below for ECM organization (−1.30) and collagen biosynthesis (−1.68), so the 30 mJ/15% program is matrix turnover, not deposition.

Burn-relevant cross-map

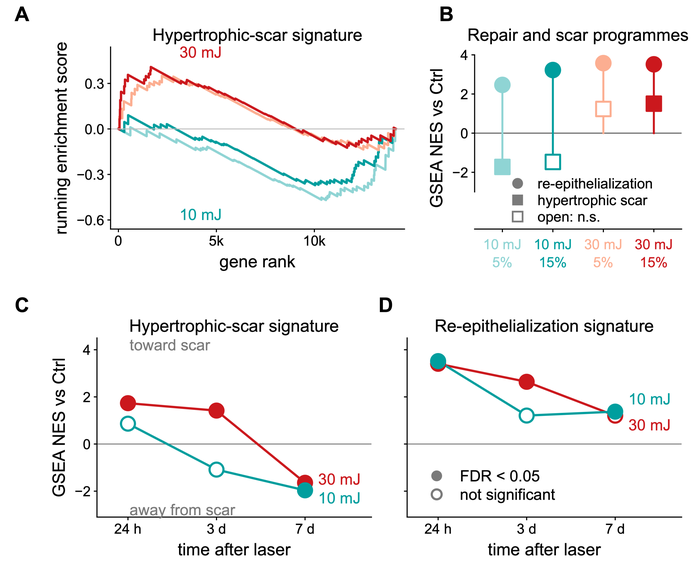
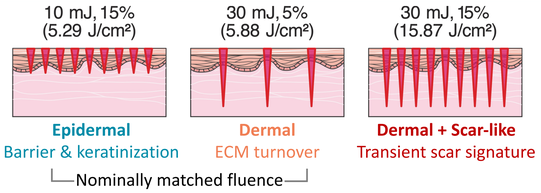


Figure 3 — The shift toward the human hypertrophic-scar transcriptome is high-energy-specific and transient; every setting initiates repair. GSEA of each setting vs its control against a hypertrophic-scar signature derived from GSE188952 (hypertrophic vs normotrophic, 54 genes) and a re-epithelialization signature (Liu et al. 2025). Filled, FDR < 0.05. (**A**, **B**) 24 h, four settings, n = 4 donors (5 for 30 mJ/15%). (**A**) Scar running enrichment: both 30 mJ arms rise, both 10 mJ arms fall, no crossing. (**B**) Scar (squares) vs re-epithelialization (circles): re-epithelialization engaged in every arm (NES +2.47 to +3.58), scar only at high energy — +1.51 at 30 mJ/15% (FDR 0.022), −1.73 at 10 mJ/5% (FDR 0.005), the other two n.s. (−1.47, +1.24). (**C**, **D**) Longitudinal, 15%-density arms, n = 3 donors. (**C**) Scar at 30 mJ engaged at 24 h (+1.73, FDR 0.003) and 3 d (+1.42, FDR 0.039), reversed by 7 d (−1.64, FDR 0.013); at 10 mJ never engaged (+0.86, −1.09), away by 7 d (−1.96, FDR 0.0004). (**D**) Re-epithelialization engaged in both arms at 24 h (+3.40, +3.52), decaying thereafter. The signature contains no collagen genes: a positive NES marks an early adhesion/remodeling state, not deposition.

4. Conclusions



- **Delivery pattern, not dose** — matched fluence, two programs: epidermal barrier at 10 mJ/15%, dermal matrix at 30 mJ/5%
- **Pulse energy selects the compartment** — at 15% density all 8 discriminating Reactome sets separate the arms (FDR ≤ 0.021)
- **Turnover, not deposition** — collagen biosynthesis is below control in every arm; qPCR *COL1A1* peaks at 3 d, baseline by 7 d
- **Scar risk is high-energy-specific and transient** — engaged only at 30 mJ, reversed by 7 d; every setting engages re-epithelialization

5. Why this matters

Burn injury is a leading cause of combat casualty morbidity, and hypertrophic scarring and contracture drive its long-term cost: reconstructive burden, delayed return to duty, medical separation. Fractional CO_2 laser is frontline rehabilitation, yet parameters are chosen by experience.
This is the **first transcriptional map linking delivery parameters to healing programs**, and it shows **geometry steers the response**.
It gives a clinical trial now under IRB review a mechanism to test rather than describe: matched fluence, varied geometry, paired contralateral sites, with *MMP1*, *LAMC2* and *COL1A1* as readout and 1-month follow-up — the timepoint no *ex vivo* model reaches, and the one that separates transient remodeling from consolidating fibrosis.

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References

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