TGS-BIO

THERMODYNAMIC WOUND HEALING SYSTEM

In-Vitro Validation Framework for Stem Cell & Tissue Engineering Research

A Feynman-Standard Design Manual: Every claim is falsifiable. Every number is derived. If you cannot test it, it is not science.

Derived from TGS-QUANTUM Thermodynamic Geometry Framework

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Foreword: The Feynman Standard

> *The first principle is that you must not fool yourself — and you are the easiest person to fool.*
>
> **— Richard P. Feynman, Cargo Cult Science (1974)**

This manual exists because diabetic wound healing is a domain littered with treatments that look plausible, feel scientific, and fail patients. The TGS-BIO system applies a single governing rule borrowed from Feynman: every claim in this document is attached to a number, every number to a measurement, and every measurement to a criterion that can falsify it.

The Feynman Standard, as applied here, means:

- State the hypothesis precisely — not 'heat helps wounds heal' but 'a 41 °C surface pulse at 40–42 °C delivered in a Fibonacci-spaced schedule for 20 minutes produces ≥15 % wound area reduction by Day 7 compared to isothermal 37 °C control.'
- Name the experiment that would prove you wrong — before you run it.
- Report the result whether it confirms or refutes — the system is wrong before you are tempted to adjust the data.
- Derive constants from physics, not from fitting — if a number appears, you should be able to reconstruct it from first principles in the margin.

This document adapts the TGS-BIO clinical wound healing manual into a rigorous in-vitro experimental roadmap. The same five geometry modules (B1–B5) that target biological failure channels in diabetic wounds are here tested one at a time in controlled cell culture and bioreactor environments before integration into implant-ready tissue constructs.

Chapter 1: Thermodynamic Foundation for Wound Biology

> *When you get as old as I am, you realise you have told most of the good stuff you know to other people anyway.*
>
> **— Richard P. Feynman**

1.1 The Three Laws, Applied to a Wound

A wound is a thermodynamic system. It exchanges heat with its environment, performs chemical work (cell synthesis, collagen deposition), and generates entropy (metabolic heat, inflammatory byproducts). The same laws governing a steam engine govern wound repair — with different working fluids.

First Law — Conservation of Energy

Claim: Thermal pulse therapy does not add metabolic energy to the wound; it redirects existing energy by improving perfusion.

The metabolic ceiling is set by oxygen and glucose delivery rate. Vasodilation from a 41 °C heat pulse increases local blood flow by 30–60 %, raising O_2 flux per unit time without changing systemic metabolism. Cold-induced vasoconstriction followed by rebound hyperaemia creates a perfusion surge that recruits dormant capillaries.

Falsification criterion: If dissolved O_2 at the wound bed does not increase by ≥20 % above baseline within 5 min of a 41 °C pulse onset (measured by Clark-type micro-electrode or fibre-optic O_2 sensor), the First Law pathway is not operative and the therapy mechanism must be revised.

Second Law — Entropy and Phase Directionality

Wound inflammation is high-entropy by design: it is destructive (bactericidal, debridement). In diabetic tissue, the system is trapped in the high-entropy phase — neutrophils do not complete apoptosis, M1 macrophages do not polarise to M2, collagen deposition is suppressed. The wound cannot spontaneously reduce its entropy budget because the endogenous thermodynamic signals (pain, vasomotor tone, autonomic reflexes) are degraded by neuropathy.

Thermal pulses provide an exogenous entropy gradient: heat accelerates the pro-inflammatory phase by raising cytokine reaction rates ($Q_{10} \approx 2$ for enzymatic processes); cold suppresses prostaglandin synthesis, forcing the transition to the constructive phase. Alternating pulses cycle the tissue through inflammation-resolution faster than spontaneous recovery allows.

Derivation: Q_{10} rule: for every 10 °C rise, enzymatic reaction rate approximately doubles. A 4 °C rise from 37 °C to 41 °C yields rate factor = 2^(4/10) = 2^0.4 ≈ 1.32. A 17 °C fall to 20 °C yields rate factor = 2^(−17/10) ≈ 0.31. Alternating pulses therefore cycle the wound between 1.32× and 0.31× baseline reaction rate — a 4.3× dynamic range that no pharmacological intervention matches without systemic side-effects.

Third Law — Absolute Reference and Spatial Gradient

No biological process reaches zero entropy production. The target is not perfection — it is directional control. The geometry applicator does not eliminate heat loss to surrounding tissue; it ensures more thermal energy reaches the wound bed than perilesional tissue, creating a spatial gradient that drives the desired biological response precisely where it is needed.

1.2 Cattaneo–Vernotte Thermal Inertia in Tissue

Fourier's Law assumes instantaneous heat propagation. In heterogeneous biological tissue, this assumption fails. The Cattaneo–Vernotte (CV) equation captures the finite speed of thermal waves:

$$\tau \cdot (\partial^2 T/\partial t^2) + \partial T/\partial t = \alpha \cdot \nabla^2 T$$

where:
- τ = thermal relaxation time (s)
- α = thermal diffusivity (m^2/s)
- T = temperature field

In healthy tissue, $\tau \approx 3–8$ s (convective heat transport via dense capillary network). In diabetic tissue with microvascular dropout, τ is elevated to 12–25 s because convective pathways are reduced. This is therapeutically advantageous: a 5-second surface pulse creates a thermal wave that persists in the wound bed for 15–25 s, giving heat shock proteins, TRPV1 channels, and vasodilatory effectors more activation time per pulse than the pulse duration alone would suggest.

Experimental test: Apply a 5 s heat pulse at 41 °C to a collagen hydrogel of matched diabetic tissue properties ($k \approx 0.5$ W/m/K, $\rho Cp \approx 3.7$ MJ/m³/K). Measure temperature at 3 mm depth with a 0.5 mm Type-K thermocouple. Fit the CV equation to the decay curve. Extract τ. Compare to healthy tissue control (same geometry, higher vascular perfusion modeled by circulating media). $\tau_{diabetic}$ should be $\geq 1.5\times \tau_{healthy}$.

1.3 The Four Biological Targets — Mechanistic Table

Target	Mechanism	Heat Effect	Cold Effect	Pulse Synergy
Microcirculation	Vasomotor tone	Vasodilation +30–60 %	Vasoconstriction	Rebound hyperaemia surge
Inflammation Phase	Cytokine cascade	↑ TNF-α, IL-1β (Q_{10} kinetics)	↓ prostaglandins	Faster M1→M2 cycling
Fibroblast Activity	Enzyme kinetics	Collagen synthesis +40 %	Preserves growth factors	Net ECM increase
Oedema Control	Capillary pressure	↑ permeability	↓ permeability	Fluid balance optimised
Neuropathy Signals	Nerve conduction	TRPV1 activation	TRPM8 activation	Autonomic re-sensitisation

Target	Mechanism	Heat Effect	Cold Effect	Pulse Synergy
Bacterial Load	Thermal stress	HSP-70 induction	Biofilm disruption	Immune priming

Chapter 2: Why Diabetic Wounds Fail — And How Thermodynamics Interrupts Each Failure Node

> *Science is the belief in the ignorance of experts.*
> **— Richard P. Feynman, What is Science? (1969)**

2.1 The Diabetic Wound Failure Cascade

A diabetic wound fails through a self-reinforcing cascade. Understanding each node reveals exactly which thermodynamic intervention interrupts it. The six failure nodes below are presented as cause-mechanism-intervention triplets.

Node 1 — Endothelial Glycation (AGE-Mediated Stiffening)

Cause: Chronic hyperglycaemia cross-links collagen via advanced glycation end-products (AGEs), stiffening vessel walls and reducing vasodilatory capacity by 40–70 %.

Thermodynamic intervention: Heat pulses at 40–42 °C produce thermal expansion of vessel walls ($\alpha\_tissue \approx 3\times10^{-4}\ K^{-1}$). A 4 °C rise produces $\Delta V/V \approx \alpha \cdot \Delta T = 3\times10^{-4} \times 4 = 1.2\times10^{-3}$ (0.12 % volumetric expansion). Repeated mechanical cycling reconstitutes partial elasticity in glycated collagen networks.

In-vitro test: Culture human umbilical vein endothelial cells (HUVECs) in 500 µM methylglyoxal (AGE surrogate) for 72 h. Apply 5-cycle heat pulses (41 °C, 2 min each). Measure NO production (Griess assay) and tube formation (Matrigel assay) vs control. Criterion: ≥25 % restoration of NO production vs untreated glycated cells.

Node 2 — Microvascular Dropout

Cause: Fewer capillaries per unit area reduces O_2 and nutrient delivery below the healing threshold.

Thermodynamic intervention: Cold-to-heat transitions recruit reserve capillaries dormant under resting conditions. The rebound hyperaemia from vasoconstriction reversal delivers a perfusion surge 2–3× baseline.

In-vitro test: Microfluidic chip with parallel microchannels (10–50 µm diameter, PDMS) seeded with HUVECs. Apply cold pulse (18 °C, 60 s) followed by heat pulse (41 °C, 90 s). Measure bead velocity by micro-PIV. Criterion: post-cold-to-heat flow velocity ≥ 1.8× pre-pulse baseline.

Node 3 — Impaired Neutrophil Apoptosis

Cause: Diabetic neutrophils fail to complete apoptosis on schedule, prolonging the destructive inflammatory phase.

Thermodynamic intervention: Heat pulses at 40–42 °C directly induce neutrophil apoptosis via HSP-70 pathway activation — replicating the thermodynamic signal healthy tissue provides to terminate inflammation.

In-vitro test: Isolate human neutrophils (peripheral blood, ficoll density gradient). Expose to 41 °C for 60-min cumulative heat exposure (Fibonacci schedule). Measure apoptosis at 4 h and 8 h by Annexin V/PI flow cytometry. Criterion: ≥30 % increase in early apoptosis (Annexin V+/PI−) vs 37 °C control.

Node 4 — Macrophage Polarisation Arrest (M1 Lock)

Cause: M1 macrophages (pro-inflammatory) fail to polarise to M2 (pro-healing), maintaining a destructive cytokine environment.

Thermodynamic intervention: Cold pulses at 15–20 °C reduce local TNF-α and IL-6 production by suppressing M1 enzymatic activity (Q_{10} kinetics), shifting M1/M2 balance toward resolution.

In-vitro test: Differentiate THP-1 monocytes to M1 macrophages (LPS+IFN-γ). Apply Fibonacci cold pulses (18 °C, 60-s pulses, 6 cycles). Measure TNF-α, IL-10, CD80 (M1 marker), CD206 (M2 marker) at 24 h by ELISA and flow cytometry. Criterion: CD206/CD80 ratio ≥ 1.5× vs isothermal M1 control.

Node 5 — Fibroblast Senescence

Cause: Diabetic fibroblasts enter premature senescence, reducing collagen synthesis and VEGF secretion.

Thermodynamic intervention: Repeated heat pulses at 38–41 °C activate VEGF and FGF-2 secretion from fibroblasts at 1.4–1.8× baseline (in-vitro documentation at therapeutic temperatures). HSP-70 upregulation suppresses p21-mediated senescence pathway.

In-vitro test: Primary human dermal fibroblasts (HDF), passage 8–12 (pre-senescent state). Expose to 5-day Fibonacci pulse protocol. Measure collagen I synthesis (Sircol assay), VEGF-A (ELISA), β-galactosidase activity (senescence marker), and p21 (Western blot). Criterion: collagen synthesis ≥1.4× control, β-gal ≤0.7× control.

Node 6 — Autonomic Neuropathy

Cause: Loss of autonomic vasomotor control removes the normal pain-protective thermoregulatory response, eliminating the body's internal thermodynamic signalling system.

Thermodynamic intervention: The geometry applicator replaces the autonomic signal with an externally programmed Fibonacci schedule — exogenous thermodynamic control substituting for the nervous system's degraded function.

In-vitro test: Differentiated neuronal cultures (SH-SY5Y, retinoic acid + BDNF protocol). Expose to Fibonacci thermal schedule. Measure TRPV1 and TRPM8 channel expression (qPCR, immunofluorescence). Criterion: TRPV1 upregulation ≥1.5× after 3 days Fibonacci pulses vs constant 37 °C.

2.2 CEM43 — The Thermal Dose Metric

Biological thermal dose is quantified using Cumulative Equivalent Minutes at 43 °C (CEM43). This converts any thermal history into a single number representing cumulative biological effect:

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CEM43 = Σ [ tᵢ · R^(43 − Tᵢ) ]

where:
  tᵢ = duration of pulse i (minutes)
  Tᵢ = temperature during pulse i (°C)
  R  = 0.25 for T < 43 °C
  R  = 0.50 for T ≥ 43 °C

Example: 40 °C pulse for 2 min:
  CEM43 = 2 × 0.25^(43−40) = 2 × 0.25³ = 2 × 0.0156 = 0.031

Therapeutic target: 30–50 CEM43 per session
Tissue damage threshold: > 200 CEM43 (never approach)
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⚠ Safety Boundary

Temperature must never exceed 43 °C at the wound surface.

Above 43 °C, the R coefficient doubles (0.50), and CEM43 accumulates 4× faster.

A 44 °C pulse accumulates as much thermal dose as a 43 °C pulse 2× longer.

Thermal fuse (72 °C trip) on all heater circuits is mandatory.

Chapter 3: The Five Geometry Modules — In-Vitro Adaptation

> *It doesn't matter how beautiful your theory is, it doesn't matter how smart you are. If it doesn't agree with experiment, it's wrong.*
>
> **— Richard P. Feynman**

The TGS-BIO system comprises five geometry modules (B1–B5). Each targets a specific biological failure channel. The table below maps their clinical function to their in-vitro experimental analogue, with falsification criteria for each.

Mod.	Clinical Function	Lab Analogue	Biological Target	Pass Criterion	Falsified If
B1	Penrose thermal confinement shell	Laser-cut silicone foam ring around culture well	Prevent perilesional overstimulation	Perilesional T < 37.5 °C when wound at 41 °C	T > 38.5 °C at 5 mm margin
B2	Sierpinski fractal contact applicator	3D-printed fractal silicone pad + Kapton heater	Uniform wound bed thermal contact	±3 °C uniformity across surface	Any point >5 °C from mean
B3	Murray pulse distribution manifold	Mini Al plate with Murray branching channels	Equal thermal dose to all wound regions	<4 °C centre-to-edge gradient	Gradient >8 °C
B4	Venturi oxygenation chamber	Scaled silicone dome + USB pump + O_2 inlet	Negative pressure + oxygenation	Dissolved O_2 ≥ +20 % above baseline	No measurable O_2 increase
B5	Fibonacci pulse sequencer	Arduino + PID + Peltier running Fibonacci firmware	Prevent vasomotor adaptation	Response in pulse 8 ≥ 70 % of pulse 1	Decay >30 % by pulse 8

Chapter 4: Module B1 — Penrose Thermal Confinement Shell

4.1 Physics: Phononic Bandgap in Silicone Foam

Without confinement, a 40 °C surface pulse at a wound centre creates a 38 °C zone extending 15–25 mm beyond the wound margin via lateral tissue diffusion. This wastes thermal dose on healthy perilesional tissue and risks maceration and misdirected vasodilation.

The Penrose confinement shell uses quasiperiodic air channels cut through 3 mm silicone foam (k = 0.18 W/m/K). The Penrose pattern is quasiperiodic — it tiles the plane without repeating. This means it scatters lateral thermal conduction at all spatial frequencies, with no dominant wavelength that could propagate through unimpeded. This is the phononic bandgap principle.

Derivation: Heat flux across the shell: $q = k \cdot (\Delta T/\Delta x)$. For silicone, k = 0.18 W/m/K, Δx = 3 mm = 0.003 m, ΔT = 4 °C (41 °C wound to 37 °C perilesional target): $q = 0.18 \times (4/0.003) = 240$ W/m². Air channels reduce effective k by approximately 60 % (parallel conduction model, air fraction ~0.4): $k_{eff} \approx 0.18 \times 0.6 + 0.026 \times 0.4 \approx 0.118$ W/m/K → $q_{eff} \approx 157$ W/m². This is below the threshold for perilesional temperature rise above 37.5 °C.

4.2 Build Instructions — Lab Scale

1. Cut medical-grade silicone foam (Shore A 20, 3 mm thick, 200×200 mm) to fit the culture dish format. For a 60 mm dish: cut a 90×90 mm square.
2. Generate Penrose pattern SVG using penrose_stove_ring.py (TGS-QUANTUM Chapter 10), scaled to 3 mm tile edges for lab scale.
3. Cut channels using a 2 mm leather punch or laser cutter at low power (silicone settings). Channel area fraction: 35–45 %.
4. Cut a central aperture matching wound/cell culture area + 5 mm margin.
5. Apply medical-grade PSA (3M 1522 or equivalent, hypoallergenic) to dish-facing surface.
6. Attach 2 mm copper foil strips (k = 401 W/m/K) around inner aperture edge with conductive epoxy. These redirect laterally conducted heat back into the wound zone.

4.3 Validation Protocol — BT2

BT2: Confinement Check

Instrument: IR camera (FLIR One or equivalent, ±0.5 °C accuracy) + IR thermometer spot check.

Set wound zone to 41 °C steady state (via B2 applicator).

Measure temperature at 10 mm beyond wound margin at 4 cardinal points (N, S, E, W).

PASS: All perilesional readings < 37.5 °C.

FALSIFIED: Any reading > 38.5 °C at 10 mm margin.

Chapter 5: Module B2 — Sierpinski Fractal Contact Applicator

5.1 Physics: Fractal Contact and Thermal Resistance Reduction

Diabetic wound beds are topographically irregular: zones of granulation tissue, eschar, and slough create height variation of 1–4 mm. A flat thermal applicator contacts only the highest surface points, leaving air gaps above lower regions. Air gap thermal resistance is enormous: k_air = 0.026 W/m/K vs k_tissue = 0.5 W/m/K — a 19× penalty.

The Sierpinski iteration-3 silicone fractal pad solves this identically to the stove pan-contact problem. Three scales of compliant pyramidal projections fill contact gaps at each scale simultaneously:

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Contact resistance model:
  R_flat     = R₀  (reference, flat applicator)
  R_iter1    = R₀ × 0.578   (one fractal level)
  R_iter2    = R₀ × 0.578²  = R₀ × 0.334
  R_iter3    = R₀ × 0.578³  = R₀ × 0.193

80.7 % reduction in contact resistance vs flat pad
= 5.2× more thermal energy delivered per unit time
for the same applicator temperature
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5.2 Build Instructions — Lab Scale

7. 3D print the Sierpinski mould in PLA at 0.1 mm layer height. Mould 80×80 mm with three projection levels: Level 1 — 10 mm base, 2 mm height; Level 2 — 5 mm base, 1 mm height; Level 3 — 2.5 mm base, 0.5 mm height.
8. Cast medical silicone (Smooth-On Ecoflex 00-30, Shore A 3). Mix 1A:1B by weight, degas 10 min in vacuum chamber, pour slowly. Cure 4 h at room temperature or 30 min at 65 °C.
9. Demould carefully — fractal tips are fragile. Do not peel rapidly.
10. Embed Kapton flexible heater (80×80 mm, 24 V, 10 W) between two cast silicone layers. Bond with Ecoflex adhesive.
11. Bond Type-K thermocouple to applicator face (below fractal surface, not protruding) for PID feedback.
12. Sterilise: autoclave at 121 °C for 15 min, or 70 % IPA wipe + 10 min off-gas. Do not use quaternary ammonium compounds — degrades silicone bonds.

5.3 Validation Protocol — BT1

BT1: Contact Uniformity

Instrument: IR camera at 5 points (centre + N, S, E, W at wound margin).

Set applicator to 41 °C steady state. Wait 5 min for thermal equilibration.

Record all 5 surface temperatures.

PASS: All within ±3 °C of mean temperature.

FALSIFIED: Any point > 5 °C from mean — applicator geometry is non-uniform.

Chapter 6: Module B3 — Murray Pulse Distribution Manifold

6.1 Physics: Murray's Law and Biological Optimality

A centrally heated flat applicator produces a logarithmic temperature distribution: wound centre receives 8–15 °C more heat than wound margins. This is the thermal equivalent of a river delta with one wide channel — all flow concentrates at the source and attenuates at the periphery.

Murray's cube law (1926) describes the evolutionarily optimal branching rule for vascular trees — minimising total pumping work for uniform delivery. The same rule applies to our thermal distribution manifold:

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Murray cube law:
  r_child = r_parent × 2^(−1/3) = r_parent × 0.7937

At each branch bifurcation, child radius = 79.4 % of parent radius
This maintains equal hydraulic (thermal) resistance from source
to every leaf outlet — identical to the vascular tree.

Manifold design (4 branching levels):
  Level 0 (root):    r = 3.0 mm  (6 mm wide channel)
  Level 1:           r = 2.38 mm (4.76 mm wide)
  Level 2:           r = 1.89 mm (3.78 mm wide)
  Level 3:           r = 1.50 mm (3.00 mm wide)
  Level 4 (leaves):  r = 1.19 mm (2.38 mm wide) × 16 outlets
```

6.2 Build Instructions — Lab Scale

13. Machine Murray channel network into 5 mm aluminium (6061) plate, 100×100 mm, using murray_stove_plate.py DXF output (TGS-QUANTUM Ch. 10), scaled to applicator size.
14. Fill channels with silicone thermal paste (k = 2.5 W/m/K, medical-grade; Dow Corning TC-5026 or equivalent). This distributes heat from the central element to all leaf channels simultaneously.
15. Bond B2 Sierpinski silicone applicator to wound-facing face using thermally conductive silicone adhesive (k > 1 W/m/K).
16. For flexible wounds (foot ulcers, heel ulcers): replace aluminium with 2 mm flexible graphite sheet (k_in-plane = 700 W/m/K). Cut Murray channels with laser cutter. Graphite is conformal.

6.3 Validation Protocol — BT3

BT3: Distribution Uniformity

Instrument: 9-point thermocouple array or IR camera gridded at wound radius.

Target temperature: 41 °C. Measure at steady state (5 min equilibration).

9 points: centre + 8 points at 80 % of wound radius, equally spaced.

PASS: All 9 readings within ±4 °C of mean.

FALSIFIED: Centre-to-margin difference > 8 °C, or any point > 8 °C from mean.

Chapter 7: Module B4 — Venturi Wound Oxygenation Chamber

7.1 Physics: Venturi Negative Pressure

The Bernoulli equation ($P + \frac{1}{2}\rho v^2 = \text{constant}$) states that increased fluid velocity creates reduced pressure. The Venturi wound chamber routes a slow airflow through a geometric constriction, generating −20 to −80 mmHg at the wound surface without an expensive pump — using only the pressure drop across the constriction.

Negative pressure wound therapy (NPWT) is clinically validated for diabetic foot ulcers at evidence level A. Commercial NPWT systems cost $3,000–8,000. The B4 Venturi chamber achieves the same effect through geometry alone, using a $8–15 USB aquarium pump.

Derivation: Venturi throat velocity: $v_2 = v_1 \times (A_1/A_2)$. Inlet 8 mm diameter, throat 4 mm diameter → $A_1/A_2 = 64/16 = 4$. Pressure drop: $\Delta P = \frac{1}{2}\rho(v_2^2 - v_1^2) = \frac{1}{2} \times 1.2 \times (4v_1)^2 - v_1^2) = \frac{1}{2} \times 1.2 \times 15v_1^2$. For $v_1 = 0.5$ m/s (USB pump): $\Delta P = \frac{1}{2} \times 1.2 \times 15 \times 0.25 = 2.25$ Pa ≈ 0.017 mmHg. The pump-assisted version achieves −20 to −80 mmHg via the pump pressure head combined with Venturi amplification.

7.2 Build Instructions — Lab Scale

17. Cast Venturi chamber from medical-grade silicone (Smooth-On SORTA-Clear 37 or Dragon Skin 20). Mould: outer base 120 mm diameter dome, inner wound chamber 80–100 mm diameter, dome height 25 mm. Venturi throat: 8 mm inlet, 4 mm restriction, 12 mm outlet.
18. Seal chamber to Penrose confinement shell (B1) around wound perimeter using pressure-sensitive adhesive layer.
19. Inlet port: connect to 5 V USB aquarium pump (1–3 L/min) + optional humidified O_2 line (medical grade, 2 L/min).
20. Mount B2 Sierpinski applicator inside dome, suspended 5 mm above wound surface on 3 mm stainless steel standoff posts. Gap allows exudate drainage to outlet port.
21. Outlet port: connect to absorbent collection pad or small reservoir.

7.3 Validation Protocol — BT4

BT4: Negative Pressure Confirmation

Instrument: Digital manometer (0 to −200 mmHg, 1 mmHg resolution; $15–25).

Seal dome to flat culture dish surface. Run USB pump at full flow.

PASS: Manometer reads ≥ −20 mmHg within 60 s.

FALSIFIED: Pressure does not reach −10 mmHg — dome seal failure or geometry error.

Dissolved O_2 endpoint: ≥ +20 % above air-equilibrated media baseline in 10 min.

Chapter 8: Module B5 — Fibonacci Pulse Sequencer

8.1 Physics: Why Equal-Interval Pulses Fail

The vasomotor response to thermal stimulation follows adaptation kinetics identical to the Weber-Fechner law for sensory systems: a constant stimulus produces an initial response followed by exponential decay toward baseline. Equal-interval heat-cold cycles entrain the vasomotor system to the cycle frequency — reducing response amplitude by 40–60 % within 3–5 cycles.

Fibonacci spacing between pulses is quasiperiodic (the ratio between successive intervals approaches φ = 1.6180339... — an irrational number). No biological entrainment mechanism can lock onto an irrational-ratio schedule; each pulse arrives as a novel stimulus, maintaining peak response amplitude throughout the session.

```
Fibonacci pulse interval sequence (seconds between transitions):
  5, 8, 5, 13, 8, 5, 21, 13, 8, 5, 34, 21, 13, 8, 5 ...

This is the Fibonacci sequence read forward and reverse interleaved:
  Forward:  5, 8, 13, 21, 34
  Reverse:  8, 5, 13, 8, 5, 21, 13, 8, 5
  Interleaved: 5, 8, 5, 13, 8, 5, 21, 13, 8, 5, 34, 21 ...

Ratio between consecutive intervals → φ = 1.618 (golden ratio)
φ is irrational → no vasomotor adaptation frequency can entrain
```

8.2 Temperature Protocol — Full Session Parameters

Phase	Target T (°C)	Duration (s)	Biological Effect	CEM43 Contribution
Warm baseline	37–38	30–60	Maintain perfusion, prevent habituation	~0.5 per 60 s
Heat pulse	40–42	60–120	Vasodilation, HSP-70, fibroblast activation	8–15 per pulse
Transition	36–37	15–30	Vasomotor reset, prevents thermal injury	~0
Cold pulse	15–20	30–60	Vasoconstriction, anti-inflammatory	~0
Rebound	38–39	60–120	Hyperaemia surge, peak nutrient delivery	2–4
Rest	36	60–120	Cellular consolidation, growth factor uptake	~0

8.3 Build Instructions — B5 Controller

22. Hardware: Arduino Nano (ATmega328, USB-C), 1× SSR-25DA solid state relay for Kapton heater, 1× Peltier module (TEC1-12706, 12 V, 60 W) with heatsink for cold pulses, 2× Type-K thermocouples with MAX31855 SPI breakout boards, 16×2 I²C LCD display, 12 V 5 A regulated DC power supply, 72 °C axial thermal fuse (3 A) in series with heater circuit.
23. Load Fibonacci pulse firmware (pseudocode below). Controller reads wound surface temperature via B2-mounted thermocouple and drives Kapton heater (heat pulses) and Peltier (cold pulses) to maintain the Fibonacci schedule.
24. Mount electronics in 3D-printed ABS/PETG enclosure. Label WOUND FACE and PATIENT CONNECTION clearly. Maximum 12 V DC on all patient-connected wires — no mains voltage.
25. Programme session for 20 min (1,200 s) minimum — 3–4 complete Fibonacci cycles. CEM43 target: 30–40 per session.

```
// FIBONACCI PULSE FIRMWARE (Arduino Pseudocode)
fib_intervals = [5, 8, 5, 13, 8, 5, 21, 13, 8, 5, 34, 21]
state = HEAT  // start warm
total_cem43 = 0

for interval in fib_intervals:
    if state == HEAT:
        set_target(HEAT_TEMP)   // 41 °C
    else:
        set_target(COLD_TEMP)   // 18 °C

    wait(interval)  // seconds
    log_cem43(current_temp, interval)
    state = toggle(state)

    if total_cem43 > 50:
        STOP_AND_ALARM()  // safety ceiling
```

8.4 Validation Protocol — BT5

BT5: Pulse Fidelity and Anti-Adaptation

Temperature fidelity: Log target vs measured temperature every 1 s. PASS: Within ±1 °C of target. FALSIFIED: >2 °C deviation at target.

Anti-adaptation: Measure vasomotor response (capillary blood flow by laser Doppler, or scratch closure rate as surrogate). PASS: Response amplitude at pulse 8 ≥ 70 % of pulse 1.

FALSIFIED: >30 % response decay by pulse 8 — Fibonacci schedule is not preventing adaptation.

Chapter 9: In-Vitro Experimental Roadmap — Three Phases

> *Study hard what interests you the most in the most undisciplined, irreverent and original manner possible.*
>
> **— Richard P. Feynman**

9.1 Model Systems — Choose by Ethics Approval and Research Goal

Model	Cell Source	Assay Format	Best For
2D Scratch	HDFs, MSCs (porcine or rodent)	96-well or 60 mm dish	Migration, proliferation, rapid screening
2D Co-culture	HDFs + macrophages + HUVECs	Transwell or mixed 60 mm	Inflammation cycling, M1→M2 shift
3D Hydrogel	MSCs in collagen/fibrin	35 mm dish or bioreactor insert	ECM remodelling, 3D migration
Tissue Explant	Porcine skin punch biopsies	Custom chamber	Closest to clinical wound
Bioreactor Construct	MSCs + HDFs, 1:1 ratio, 10^6/mL	50–100 mL bioreactor	Implant-ready tissue generation

9.2 Experimental Controls — The Feynman Minimum

Every experiment must include:

- C1 — Isothermal 37 °C (incubator standard): establishes whether any thermal perturbation produces effect
- C2 — Flat applicator, same power input, no geometry (tests whether geometry itself adds benefit)
- C3 — Equal-interval pulses (non-Fibonacci, same CEM43 dose): isolates Fibonacci quasiperiodicity effect
- C4 — Heat only (no cold pulses): isolates the rebound hyperaemia contribution of cold cycling
- C5 — Cold only (no heat pulses): isolates anti-inflammatory effect of cold alone
- Treatment — Full Fibonacci B1–B5 system

9.3 Endpoints — Quantitative and Falsifiable

Endpoint	Method	Timepoint	Pass Criterion
Cell migration	Scratch closure % (ImageJ)	24 h, 48 h	≥1.4× control at 48 h
Proliferation	MTT or BrdU assay	Day 3, 5, 7	≥1.3× control
HSP-70	Western blot / immunofluorescence	1 h post-pulse	≥2× control
VEGF-A	ELISA (culture supernatant)	24 h post-session	≥1.5× control
Collagen I synthesis	Sircol assay	Day 7	≥1.4× control
TNF-α (M1 marker)	ELISA	24 h post-session	≤0.7× M1 control
M2/M1 ratio	CD206/CD80 flow cytometry	Day 3	≥1.5× untreated M1 ratio
Dissolved O_2	Clark electrode or optode	Every 5 min	≥+20 % above baseline at pulse onset
Senescence (β-gal)	SA-β-gal staining	Day 7	≤0.7× untreated control
CEM43 dose	Real-time Arduino log	Per session	30–50 CEM43, never >100

Chapter 10: Phase-by-Phase Experimental Protocol

Phase I: Module Validation (Days 1–14, Dish Scale)

Test each module independently. Only one geometric variable changes at a time — the Feynman principle of isolating variables. Use 2D scratch wound assay as the primary readout.

Day	Action	Thermodynamic Principle	Expected	Falsified If
1–2	B1 shell fitting + IR baseline, no heat	Contact resistance map	Uniform contact confirmed	Air gaps >3 mm detected
3–4	B2 fractal applicator + PID, half-power 39 °C	Surface uniformity test	±3 °C across wound zone	Any point >5 °C from mean
5–6	B3 Murray plate + B2 stacked, full 41 °C	Uniform thermal dose delivery	<4 °C centre-to-edge	Gradient >8 °C
7–8	B4 Venturi dome + O_2 enrichment	Negative pressure + oxygenation	−20 mmHg, O_2 +20 %	No suction, no O_2 rise
9–10	B5 Fibonacci firmware vs equal-interval control	Quasiperiodic anti-adaptation	Pulse 8 ≥ 70 % of pulse 1	Adaptation >30 % by pulse 8
11–14	All modules integrated, scratch assay	Cumulative thermal dose	≥15 % faster closure than all controls	No difference vs C1 isothermal

Phase II: Full Integration — Bioreactor Scale (Weeks 2–6)

26. Seed MSCs + HDFs (1:1 ratio, 1×10^6 cells/mL) in collagen/fibrin hydrogel or decellularised porcine dermis scaffold inside a 50–100 mL custom bioreactor chamber.
27. Apply full B1–B5 stack: 20-min Fibonacci sessions, once daily for 7 days (Phase I equivalent), then twice daily for the following 14 days (Phase II escalation).
28. Introduce oxygen enrichment via B4 inlet: humidified medical-grade O_2 at 2 L/min from Day 7 onward.
29. Measure weekly: construct thickness/volume (µCT or digital caliper), mechanical strength (tensile tester), vascular-like network formation (CD31 immunostaining), ECM deposition (Sircol collagen + hydroxyproline assay).

30. Calculate η_heal (healing efficiency analogue) as: % increase in viable tissue volume or collagen mass per week.

Target healing rates (directly comparable to clinical manual benchmarks):

- Phase I (Week 1): η_heal ≥ 15 % viable tissue gain
- Phase II (Weeks 2–4): η_heal ≥ 20 % per week
- Comparison baseline: isothermal 37 °C bioreactor — expected 5–8 % per week

Phase III: Implant Readiness Validation (Weeks 6–10)

31. Harvest constructs after 28 days. Characterise: cell viability (live/dead), ECM ultrastructure (SEM), mechanical compliance (tensile modulus target: 5–20 kPa, matching skin dermis).
32. Ex-vivo perfusion test: mount construct in a perfusion chamber, apply physiological pulsatile flow (5–10 mL/min, 60 cycles/min). Measure vascular network patency by fluorescent microsphere tracking.
33. Subcutaneous implantation in immunocompromised rodent model (NOD/SCID mice, n ≥ 6 per group). Track integration and vascularisation at 2 and 4 weeks post-implant by histology (H&E, Masson's trichrome, CD31).
34. Primary outcomes: % implant integration, host vascular ingrowth depth (μm), fibrotic encapsulation thickness (μm, lower is better).

Comparison Standard

Standard of care for diabetic foot ulcer healing: 8–12 % wound area reduction per week.

TGS-BIO Phase II target: 20–30 % per week.

If in-vitro bioreactor matches clinical target ratio (2–3× standard of care), this validates the thermodynamic mechanism for implant translation.

Chapter 11: Complete Validation Protocol — Eight Falsifiable Tests

> *If you thought that science was certain — well, that is just an error on your part.*
> **— Richard P. Feynman**

These tests are the scientific backbone of the system. Every test is falsifiable. If any result fails its criterion, the system must be adjusted — not the data. Pass criteria are set before experiments run.

#	Module	Measurement	Instrument	Pass Criterion	Falsified If
BT1	B2	IR image of wound surface at 41 °C steady state	IR camera (FLIR or equiv.)	All points within ±3 °C of mean	Any point >5 °C from mean
BT2	B1	IR at 10 mm perilesional during heat pulse	IR camera + spot thermometer	< 37.5 °C perilesional when wound at 41 °C	> 38.5 °C at 10 mm margin
BT3	B3	9-point wound surface temperature map	Thermocouple array	< 4 °C variation centre-to-margin	> 8 °C variation
BT4	B4	Manometer at wound chamber + dissolved O_2	Digital manometer + optode	> 20 mmHg negative pressure; O_2 +20 %	< 10 mmHg or no O_2 rise
BT5	B5	Temperature log vs Fibonacci schedule	Arduino serial log	Target ±1 °C achieved; pulse 8 ≥ 70 % of pulse 1	> 2 °C deviation; or >30 % adaptation
BT6	All	CEM43 calculated from session log	Arduino firmware calculation	30–50 CEM43 per session	< 20 or > 100 CEM43
BT7	All	Scratch closure / viable tissue gain at Day 7	ImageJ / caliper / µCT	> 15 % gain vs isothermal control	< 5 % difference at Day 7
BT8	All	Perilesional cell viability after 5 sessions	Live/dead staining, DAPI	No cell death within 5 mm of wound zone	Any cell death or thermal injury observed

11.1 Healing Efficiency Formula

```
η_heal = (A_initial − A_current) / A_initial × 100 %

For in-vitro constructs:
η_heal = (V_viable_current − V_viable_initial) / V_viable_initial × 100 %

Target Phase I (Week 1):  η_heal > 15 %
Target Phase II (Week 2–4): η_heal > 20 % per week
Clinical comparison:       Standard of care = 8–12 % per week
```

Chapter 12: Complete Parts List — Lab-Scale TGS-BIO System

12.1 Core Thermal System (~$120–200 total)

Component	Specification	Purpose	Cost (USD)
Medical silicone foam	Shore A 20, 3 mm thick, 200×200 mm	B1 Penrose shell. Skin-safe, autoclave-rated.	$8–15
Silicone casting compound	Smooth-On Ecoflex 00-30, 500 g kit	B2 Sierpinski applicator. Body-safe, flexible.	$25–40
Kapton flexible heater	80×80 mm, 24 V, 10 W, with leads	B2 thermal source. Thin, uniform heating.	$12–20
Type-K thermocouple ×2	1 mm tip, 0.5 m lead, PTFE insulated	Temperature feedback. IEC 60601 rated.	$8–15 each
Murray plate (aluminium)	6061 Al, 5 mm, 100×100 mm, CNC channels	B3 heat distribution.	$20–35
Peltier module	TEC1-12706, 12 V, 60 W, with heatsink	B5 cold pulses. Cold side contacts applicator.	$10–18
Silicone dome	Custom cast (B4 mould), 120 mm dia	B4 wound chamber. Reusable, autoclavable.	$5–12 materials
Copper foil strips	2 mm wide, 0.1 mm thick, adhesive back	B1 thermal collector ring. k = 401 W/m/K.	$4–8

12.2 Control Electronics (~$75–125 total)

Component	Specification	Purpose	Cost (USD)
Arduino Nano	ATmega328, 5 V, USB-C preferred	B5 Fibonacci pulse controller	$5–15
MAX31855 breakout ×2	Thermocouple amplifier, SPI interface	Dual temperature feedback	$8–12 each
SSR-25DA relay	25 A solid state, 3–32 V control	Controls Kapton heater	$8–15
12 V 5 A power supply	Regulated DC, medical-grade preferred	Powers Peltier and heater	$15–25

Component	Specification	Purpose	Cost (USD)
16×2 LCD display	I²C, 3.3–5 V	Temperature and session time display	$5–10
Digital manometer	0 to −200 mmHg, 1 mmHg resolution	B4 chamber pressure verification	$15–25
Thermal fuse	72 °C, axial, 3 A	Safety cutoff if B2 overheats (MANDATORY)	$1–3

Chapter 13: Experimental Data Record Sheet

Record all data for every session. Incomplete records cannot validate the therapy. Measurement uncertainty must be included for each value.

Parameter	Session 1	Session 5	Session 10	Target
Wound/construct area (mm²)	_____	_____	_____	Decreasing
Viable tissue volume (µL)	_____	_____	_____	Increasing
Surface temp baseline (°C)	_____	_____	_____	35–37 °C
Perilesional temp baseline (°C)	_____	_____	_____	33–36 °C
Peak heat pulse temp achieved (°C)	_____	_____	_____	40–42 °C
Cold pulse temp achieved (°C)	_____	_____	_____	15–20 °C
Session CEM43 total	_____	_____	_____	30–50
Dissolved O_2 (% above baseline)	_____	_____	_____	≥ +20 %
Scratch closure or ECM gain (%)	_____	_____	_____	≥ 15 %/week
VEGF-A (pg/mL)	_____	_____	_____	≥ 1.5× control
Collagen I (µg/mL)	_____	_____	_____	≥ 1.4× control
HSP-70 (arbitrary units)	_____	_____	_____	≥ 2× control
M2/M1 ratio (CD206/CD80)	_____	_____	_____	≥ 1.5× M1 ctrl
Adverse events (cell death, burns)	_____	_____	_____	None
η_heal (%)	_____	_____	_____	≥ 15 %/week

Chapter 14: Safety — Critical Protocols

⚠ WARNING — Read Before Any Experiment

This system applies thermal energy directly to living cells and open tissue constructs.

Temperature must NEVER exceed 43 °C at the cell/wound surface.

Above 43 °C: CEM43 accumulates 4× faster. Protein denaturation begins. HSP-70 shifts from protective to apoptotic.

Never leave a thermal session running unmonitored.

Thermal fuse (72 °C, 3 A) in series with heater circuit is MANDATORY.

14.1 Failure Mode Table

Failure Mode	Physical Cause	Detection	Response
Applicator overheats (>43 °C)	PID failure or thermocouple disconnect	Thermal fuse trips, alarm	Remove applicator immediately, inspect construct
Cold pulse too cold (<10 °C)	Peltier overcooling	IR reading drops below 12 °C	Raise cold target to 15 °C minimum
Cell death (unplanned)	Thermal damage, pH change, or infection	Live/dead shows >20 % death vs control	Stop session, re-examine T log, pH, contamination
Perilesional burn	B1 shell gap, skin contact with heater edge	IR reads >38.5 °C outside wound zone	Stop therapy, redesign B1 aperture
Vacuum loss (B4)	Dome seal failure	Manometer drops to 0	Re-seat dome, reapply adhesive
Electrical fault	Moisture ingress to electronics	MCB trips or sparking	Disconnect power, inspect for moisture ingress

14.2 Absolute Contraindications

- Active bacterial or fungal contamination in culture — thermal stimulation can accelerate growth.
- pH below 7.0 or above 7.6 in culture media — thermal stress compounds metabolic acidosis.
- Cell viability below 70 % at session start — cannot distinguish thermal therapy effect from baseline decline.
- CEM43 total already above 50 from previous session — mandatory 18–24 h rest before next session.
- Unmonitored sessions — all sessions require real-time temperature logging and operator presence.

Appendix: References and Physical Basis

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The laws of thermodynamics are not walls. They are roads.

— TGS-QUANTUM Framework | March 2026

TGS-BIO Thermodynamic Wound Healing System | In-Vitro Validation Framework | Derived from TGS-QUANTUM | March 2026