

HEMPOXY MECHANICAL ONCOLOGY PLATFORM

Master Research & Development Plan
Phases I–IV: Material Science to Preclinical Efficacy

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1 EXECUTIVE SUMMARY

This Master Research & Development Plan (RDP) outlines a 18-month pathway to validate the Hempoxy Mechanical Oncology platform: a novel approach to tumor cell disruption combining hemp-fiber epoxy composite microneedles, AI-guided targeting, localized chitosan-based debris sequestration, and preclinical efficacy assessment.

1.1 Problem Statement

Conventional chemotherapy kills cancer cells rapidly, triggering tumor lysis syndrome (TLS)—a life-threatening metabolic emergency characterized by hyperkalemia, hyperphosphatemia, and hyperuricemia. Current prophylactic standard-of-care (hydration + allopurinol/rasburi-case) carries burden of fluid overload, variable renal clearance, and medication side effects. An alternative approach that controls cell lysis location and sequesters debris at the point of disruption could reduce systemic complications.

1.2 Proposed Solution

Mechanical disruption of tumor cells via AI-coordinated Hempoxy microneedle arrays coupled with pH-triggered chitosan-mediated intracellular debris sequestration. This approach:

- Treats tumors as structural anomalies subject to physical failure
- Localizes debris release (reducing systemic burden)
- Integrates real-time AI targeting for cell-type selectivity (cancer vs. normal)
- Requires no systemic pharmacologic prophylaxis

1.3 Phase Structure

Phase	Focus	Duration	Key Deliverable
I	Material characterization	Months 1–3	Property validation report
II	Tumor-on-chip integration	Months 4–6	Efficacy & selectivity report
III	AI targeting optimization	Months 7–12	ML model + targeting algorithm
IV	Preclinical efficacy	Months 13–18	Xenograft tumor study report

1.4 Success Criteria (Gate Criteria)

- **Phase I Gate:** Hempoxy tensile strength ≥ 100 MPa confirmed; chitosan binding efficiency $\geq 70\%$ for K^+ and nucleotides

- **Phase II Gate:** Tumor spheroid lysis $\geq 90\%$; selectivity (cancer vs. normal) $\geq 85\%$; systemic cytokine elevation ≤ 5 pg/mL
- **Phase III Gate:** AI classification accuracy $\geq 95\%$ on held-out cell population; targeting error $< 5\%$
- **Phase IV Gate:** Tumor regression comparable to standard chemotherapy + TLS prophylaxis; no TLS-related mortality; acceptable safety profile

2 PHASE I: MATERIAL CHARACTERIZATION & FORMULATION (Months 1–3)

2.1 Objectives

1. Validate and correct Hempoxy tensile strength specifications
2. Optimize nanoparticle reinforcement (graphene, boron nitride) to achieve ≥ 100 MPa
3. Characterize chitosan binding kinetics for intracellular debris (K^+ , nucleotides)
4. Fabricate and mechanically test microneedle prototypes
5. Establish baseline biocompatibility (hemolysis, cytotoxicity assays)

2.2 Task 1.1: Hempoxy Tensile Strength Validation (Weeks 1–4)

2.2.1 Hypothesis

Standard hemp/epoxy composite (30 vol% hemp fiber, no reinforcement) achieves tensile strength of 50–80 MPa, consistent with peer-reviewed literature. Nanoparticle addition (2 wt% graphene oxide or functionalized boron nitride) increases strength to 100–120 MPa without sacrificing biocompatibility.

2.2.2 Materials

- Hemp fiber (grade: textile, 100–300 μm diameter, Moncton supplier or equivalent)
- Epoxy resin (type: cycloaliphatic or bisphenol-A, cure agent: aliphatic amine)
- Graphene oxide nanosheets (lateral size: 1–10 μm , thickness: 0.5–2 nm)
- Boron nitride nanoplatelets (h-BN, 100 nm–1 μm , purity $> 99\%$)
- Coupling agent (3-aminopropyl-triethoxysilane for fiber-resin bonding)

2.2.3 Procedure: ASTM D638-Type IV Specimens

1. Fiber Preparation:

- Dry hemp fiber at 80 $^{\circ}\text{C}$ for 2 hours (remove moisture)
- Treat with silane coupling agent (0.5 wt% in ethanol) for 30 minutes
- Cure at 120 $^{\circ}\text{C}$ for 1 hour
- Cool to room temperature

2. Nanoparticle Dispersion (if applicable):

- Disperse graphene oxide or h-BN in epoxy resin via sonication (40 kHz, 30 min)
- Thermal annealing at 150 °C for 1 hour to reduce graphene oxide (if using GO)
- Cool before fiber incorporation

3. Composite Lamination:

- Mix epoxy + hardener at 1:1 mass ratio (or per manufacturer)
- Add treated hemp fibers: 30 vol% (fiber volume fraction determined by packing)
- Manual mixing 2 minutes; degass under vacuum 10 minutes
- Pour into aluminum mold (ASTM D638-Type IV: 10 mm × 7 mm × 120 mm)
- Cure: 24 hours at room temperature + 2 hours at 80 °C (post-cure)

4. Specimen Conditioning:

- Store specimens in 50% relative humidity, 23 °C for ≥ 40 hours before testing (ASTM D618)
- Machine tensile test specimens to final dimensions if necessary

2.2.4 Tensile Testing (ASTM D638)

- **Equipment:** Instron 5948 or equivalent (5 kN load cell); displacement rate 5 mm/min
- **Specimens per condition:** $n = 5$ (standard requirement)
- **Measured Parameters:**
 - Ultimate tensile strength (UTS): maximum load / original cross-sectional area
 - Yield strength (0.2% offset method, if visible)
 - Young’s modulus (slope of linear region, 0–0.5% strain)
 - Elongation at break (%)
 - Stress–strain curve (export raw data)

2.2.5 Acceptance Criteria

- Standard hemp/epoxy: 50–80 MPa (consistent with literature baseline)
- Graphene-reinforced hemp/epoxy: ≥ 100 MPa (gate criterion)
- h-BN-reinforced hemp/epoxy: ≥ 100 MPa (gate criterion)
- Coefficient of variation (CV) within condition: $\leq 15\%$ (acceptable variability)

2.2.6 Deliverable

Technical Report: “Hempoxy Composite Tensile Characterization (Baseline + Nanoparticle Variants)”

- Stress–strain curves for all conditions
- Summary table: mean UTS, modulus, elongation, CV
- SEM micrographs of fracture surfaces (fiber–matrix bonding assessment)
- Selected nanoparticle variant for downstream work (graphene or h-BN, justify)

2.3 Task 1.2: Microneedle Fabrication & Mechanical Characterization (Weeks 3–8)

2.3.1 Objective

Fabricate Hempoxy microneedle arrays (geometry: conical tips, 500 nm–1 μ m diameter base) and validate piercing force and buckling resistance.

2.3.2 Fabrication Protocol

Method: Lithography-Assisted Molding (LAM) or Direct Write

1. Mold Fabrication (One-Time):

- Photolithography on SU-8 photoresist (thickness: 50–100 μ m) to define conical tip geometry
- Develop mold to define inverse cone pattern (tip diameter: 500 nm, base: 5 μ m, height: 20 μ m)
- Array configuration: 100 $\times$ 100 needles (spacing: 50 μ m center-to-center)
- Silanize mold with octadecyl-trichlorosilane to enable demolding

2. Hempoxy Composite Molding:

- Mix Hempoxy (nanoparticle-reinforced variant from Task 1.1)
- Cast into silanized mold under vacuum
- Cure per Task 1.1 protocol
- Demolding at 4 °C (brittle state facilitates release)

3. Array Integration:

- Bond Hempoxy needle array to piezoelectric substrate (lead zirconate titanate, PZT) using epoxy adhesive (medical-grade)
- Electrical contact patterning: sputtered platinum electrodes (2 kV, 20 mA, 2 min)
- Wire bonding or flex circuit attachment for electrical actuation

2.3.3 Mechanical Characterization

Piercing Force Measurement (Atomic Force Microscopy):

- Mount single needle on AFM cantilever
- Approach speed: 1 μ m/s

- Target: synthetic cell membrane mimic (lipid bilayer on polyethylene terephthalate substrate)
- Measure force at penetration depth: 1 μm
- Target piercing force: 50–100 nN (literature-consistent range)
- Repeat: $n = 10$ needles from separate fabrication runs

Buckling Resistance (Optical Microscopy + Load Measurement):

- Apply axial compressive force via micromanipulator
- Measure deflection vs. applied force (0–500 nN range)
- Critical buckling load: $F_{\text{cr}} = \frac{\pi^2 EI}{(KL)^2}$
 - E = measured Young’s modulus
 - I = second moment of inertia (conical geometry)
 - K = end-condition coefficient ($K \approx 1$ for fixed–free)
 - L = needle length (20 μm)
- Target: $F_{\text{cr}} > 500$ nN (exceed expected lysis forces by $\geq 5\times$)

2.3.4 Deliverable

Technical Report: “Hempoxy Microneedle Array Characterization”

- Optical/SEM micrographs of needle array (plan and side view)
- AFM force curves (piercing force distribution, $n = 10$)
- Buckling force measurements and Euler equation validation
- Assessment: readiness for biological testing

2.4 Task 1.3: Chitosan Debris Binding Kinetics (Weeks 4–12)

2.4.1 Objective

Quantify pH-triggered chitosan binding efficiency for intracellular potassium (K^+) and nucleotides (ATP, GTP, nucleotide fragments) under physiological conditions.

2.4.2 Hypothesis

Chitosan (MW: 50–190 kDa, degree of deacetylation: $\geq 85\%$) cross-linked via genipin and coated on microneedle tips will electrostatically bind $\geq 70\%$ of released K^+ and nucleotides within 5 seconds at physiological pH (7.4) and osmolarity (310 mOsm/kg).

2.4.3 Materials

- Chitosan (medium MW, 100 kDa nominal, 85% deacetylated)
- Genipin (cross-linker; 0.5–2 mM in ethanol)
- Potassium chloride (KCl, analytical grade)
- Nucleotides: ATP, GTP, CTP, UTP (sodium salts, 99% purity)
- Simulated cytoplasmic fluid (SCF): 140 mM K^+ , 5 mM Na^+ , 2 mM Ca^{2+} , 1 mM Mg^{2+} , phosphate buffer pH 7.4
- Radiolabel standards: ^{42}K (potassium) and ^{32}P (nucleotides) for detection

2.4.4 Procedure: Binding Kinetics Assay

Part A: Chitosan Coating Optimization

1. Prepare microneedle substrates (glass slides, 100×100 array)
2. Coat with chitosan solution (1–2 mg/mL in 1% acetic acid) via dip-coating or spin-coating
3. Cross-link via genipin vapor (50 °C, 30 min) or immersion (0.5 mM genipin, 1 hour)
4. Wash: water (3 $\times$), PBS (3 $\times$), air dry
5. Characterize coating thickness via ellipsometry or profilometry (target: 1–5 μm)
6. Assess coating uniformity: SEM inspection ($n = 3$ substrate replicates)

Part B: K^+ Binding Kinetics

1. Prepare K^+ solutions in SCF: 10, 50, 140 mM (simulate intracellular to physiological range)
2. Label with ^{42}K (activity: 10 $\mu Ci/mL$)
3. Add chitosan-coated substrate to K^+ solution (volume: 1 mL per well)
4. Incubate at 37 °C; sample radioactivity at timepoints: 0, 1, 2, 5, 10, 30, 60 sec
 - Measure solution radioactivity (scintillation counter)
 - Subtract from initial to calculate substrate-bound fraction
5. Binding curve: $B(t) = B_{\max}(1 - e^{-kt})$
 - $B(t)$ = fraction bound at time t
 - $B_{\max}$ = maximum binding (saturation)
 - k = binding rate constant (s^{-1})
6. Replicate: $n = 3$ substrates per condition

Part C: Nucleotide Binding Kinetics

1. Prepare nucleotide mixture in SCF: ATP, GTP, CTP, UTP (10 mM each)
2. Label with ^{32}P (activity: 10 $\mu Ci/mL$)
3. Repeat incubation and sampling as Part B
4. Measure ^{32}P -labeled nucleotide binding kinetics
5. Distinguish binding by nucleotide type (if time permits: HPLC + radiometric detection)

2.4.5 Acceptance Criteria

- K^+ binding: $B_{\max} \geq 70\%$ at 140 mM (intracellular concentration)
- Nucleotide binding: $B_{\max} \geq 70\%$ for ATP + GTP (primary energy molecules)
- Binding kinetics: t_{90} (time to reach 90% saturation) ≤ 5 seconds
- Coating stability: no delamination after 5 binding/wash cycles

2.4.6 Deliverable

Technical Report: “Chitosan Debris Sequestration: Kinetics & Optimization”

- Binding curves (K^+ and nucleotides, all concentrations)
- Summary table: $B_{\max}$, k , t_{90} for each condition
- SEM/ellipsometry characterization of chitosan coating
- Assessment of in vivo applicability (osmolarity sensitivity, pH dependence)

2.5 Task 1.4: Biocompatibility Assessment (Weeks 6–12)

2.5.1 Objective

Validate that Hempoxy needles and chitosan coating are non-hemolytic and non-cytotoxic to normal cell lines.

2.5.2 Assays

Hemolysis Assay (ASTM F756):

- Incubate Hempoxy needles (5 mg) in fresh human or rat red blood cells (RBCs, 2% suspension in isotonic saline)
- Incubate 1 hour at 37 °C
- Measure hemoglobin release (absorbance 545 nm)
- Positive control: 1% Triton-X100 (100% hemolysis)
- Negative control: isotonic saline (0% hemolysis)
- Acceptance criterion: % hemolysis $\leq 5\%$ (ASTM F756 limit for medical devices)

Cytotoxicity Assay (MTT or LDH Release):

- Cell lines: Human fibroblasts (normal) + cancer line (e.g., HeLa or MCF-7)
- Prepare Hempoxy needle extract: incubate needles in DMEM (10% serum) for 24 hours at 37 °C
- Treat cells with needle extract (10–100% v/v, $n = 3$ replicates each)
- Measure viability at 24 h via MTT (tetrazolium reduction) or LDH release (cytotoxicity)
- Acceptance criterion: $\geq 80\%$ viability at 100% extract concentration

2.5.3 Deliverable

Technical Report: “Biocompatibility Assessment (Hemolysis + Cytotoxicity)”

2.6 Phase I Summary

Upon completion of Phase I (Months 1–3), the following must be confirmed:

1. Hempoxy tensile strength ≥ 100 MPa (nanoparticle-reinforced) — **GATE CRITERION**
2. Microneedle piercing force 50–100 nN; buckling force > 500 nN
3. Chitosan binding efficiency: K^+ and nucleotides $\geq 70\%$, $t_{90} \leq 5$ sec
4. Biocompatibility: hemolysis $\leq 5\%$; cytotoxicity $\leq 20\%$ at 100% extract

GO/NO-GO DECISION: If all criteria met, proceed to Phase II. If not, iterate Task 1.1–1.3 or reassess platform feasibility.

3 PHASE II: TUMOR-ON-CHIP INTEGRATION & EFFICACY VALIDATION (Months 4–6)

3.1 Objectives

1. Integrate Hempoxy needle array into 3D microfluidic tumor-on-chip device
2. Demonstrate $\geq 90\%$ tumor spheroid lysis via mechanical disruption
3. Quantify selectivity (cancer vs. normal cell lysis): target $\geq 85\%$
4. Measure systemic cytokine elevation (IL-6, TNF- α , IL-1 β): target ≤ 5 pg/mL
5. Validate chitosan sequestration in integrated system

3.2 Task 2.1: Tumor-on-Chip Device Design & Fabrication (Weeks 4–8)

3.2.1 Device Architecture

The tumor-on-chip consists of three integrated chambers:

1. Tumor Chamber:

- 3D hydrogel matrix (Matrigel or collagen-I, 100 μ L volume)
- Embedded tumor spheroids (primary patient-derived xenograft cells or established lines)
- Spheroid diameter: 100–300 μ m (cultivated 3–5 days prior)
- Perfusion inlet/outlet for nutrient supply during culture

2. Mechanical Disruption Chamber:

- Integrated Hempoxy needle array (100 $\times$ 100 from Phase I)
- Piezoelectric actuator substrate (PZT, frequency range: 10–200 Hz)
- Electrical control interface (frequency, amplitude, duty cycle programmable)
- Optical access for real-time microscopy

3. Co-Culture Chamber (Selectivity Control):

- Normal fibroblasts (same species, 50% of chamber volume)
- Spatially separated from tumor chamber via semi-permeable membrane (10 μ m pore, allows cytokine diffusion but not cell migration)
- Measures bystander injury (indirect damage from debris or cytokines)

3.2.2 Fabrication Protocol (Polydimethylsiloxane, PDMS)

1. Soft lithography: replica-mold chambers from SU-8 masters (channel height: 500 μm ; width: 2 mm)
2. Bond PDMS to glass coverslip via plasma oxidation
3. Prime channels with fibronectin or collagen (10 $\mu\text{g}/\text{mL}$, 1 hour)
4. Integrate Hempoxy needle array via epoxy bonding (medical-grade adhesive, low out-gassing)
5. Connect fluidic inputs/outputs via Tygon tubing (0.5 mm inner diameter)
6. Validate assembly: dye perfusion test, leak detection

3.3 Task 2.2: Tumor Spheroid Preparation & Integration (Weeks 6–9)

3.3.1 Spheroid Generation

Option A: Established Cancer Cell Lines

- Cell lines: HeLa (cervical), MCF-7 (breast), A549 (lung), or patient-derived xenograft (PDX)
- Culture 2D monolayer in standard media (DMEM + 10% FBS)
- Spheroid formation: ultra-low attachment 96-well plate (Corning Ultralow Attachment)
 - Resuspend cells at 1000 cells/well in spheroid medium (DMEM + 2% Matrigel)
 - Incubate 3–5 days at 37 °C, 5% CO₂
 - Harvest spheroids by gentle pipetting
 - Size uniformity: 100–300 μm (select via sieve or image analysis)

Option B: Primary Patient-Derived Xenograft (PDX) Cells

- Source: tumor resection or diagnostic biopsy (requires IRB approval)
- Isolate and expand cells in culture for 2–3 passages
- Form spheroids as above (typically slower growth; allow 7–10 days)

3.3.2 Spheroid Embedding in Device

1. Prepare Matrigel or collagen-I solution (dilute to 50% final concentration)
2. Collect spheroids in chilled Matrigel
3. Load into tumor chamber via syringe injection (100 μL volume)
4. Incubate 30 min at 37 $^{\circ}\text{C}$ to solidify hydrogel
5. Initiate perfusion (0.1 $\mu\text{L}/\text{min}$ flow rate) to provide nutrients
6. Allow 12–24 hours of equilibration before lysis experiment

3.4 Task 2.3: Mechanical Lysis Protocol & Real-Time Monitoring (Weeks 8–10)

3.4.1 Lysis Parameters (Speculative, Requires Optimization)

- **Frequency:** 100–200 Hz (start at 150 Hz; optimize based on efficacy)
- **Force per needle:** 50–100 nN (from Phase I characterization)
- **Duration per spheroid:** 2–5 seconds
- **Array activation pattern:** Sequential rolling-wave (activate needles in 10×10 subgrids; delay 100 ms between subgrids)
 - Rationale: localize debris release and allow chitosan binding before systemic diffusion
- **Temperature:** 37 $^{\circ}\text{C}$ (maintain via thermoelectric controller)

3.4.2 Real-Time Monitoring: Electrical Impedance Spectroscopy (EIS)

1. Integrate impedance electrodes into device (interdigitated platinum, embedded in PDMS)
2. Apply AC voltage sweep: 100 Hz–1 MHz, 0.1 V amplitude
3. Record impedance $Z(\omega)$ and phase angle θ before, during, and after lysis
4. Interpretation:
 - Cell membrane rupture $\rightarrow$ decrease in impedance (loss of membrane barrier)
 - Intact tumor spheroid: high impedance (cell clusters highly polarizable)
 - Lysed debris: low impedance (conductivity increases)
5. Data acquisition: ≥ 1 Hz temporal resolution

3.4.3 Microscopy Documentation

- Bright-field microscopy (10× or 20× objective) at 1 fps (time-lapse video)
- Fluorescence imaging (optional): live/dead stain (calcein-AM/propidium iodide) at 0, 1, 3, 5 min post-lysis
- Morphology assessment: spheroid integrity, membrane rupture, debris morphology

3.4.4 Acceptance Criteria

- Tumor spheroid lysis: $\geq 90\%$ cell death (by live/dead stain) at optimized parameters
- EIS signature: measurable impedance change correlating with microscopy observations
- Consistency: lysis efficiency $CV \leq 15\%$ across $n = 5$ spheroids

3.5 Task 2.4: Selectivity Assessment (Cancer vs. Normal) (Weeks 9–12)

3.5.1 Objective

Quantify targeting selectivity: ratio of cancer cell lysis to normal fibroblast lysis.

3.5.2 Experimental Design

Setup A: Mono-Culture Selectivity

- Run lysis protocol on tumor spheroids (from Task 2.3)
- Separately: run identical lysis protocol on normal fibroblast spheroids (same size, cultivated identically)
- Compare lysis efficiency (cancer vs. normal)
- Selectivity Index (SI) = $\frac{\text{Cancer lysis (\%)}}{\text{Normal lysis (\%)}}$
- Target: SI ≥ 4 (at minimum 4:1 cancer:normal ratio)

Setup B: Co-Culture Selectivity (Bystander Effect)

- Embed tumor spheroid in chamber; normal fibroblasts in adjacent chamber (via semi-permeable membrane, 10 μm pore)
- Run lysis protocol on tumor spheroid
- Measure fibroblast viability post-lysis via:
 - Live/dead stain (calcein-AM/PI)
 - LDH release assay (fibroblast lysate in supernatant)
- Quantify indirect injury: % fibroblast lysis in co-culture condition
- Comparison: mono-culture lysis (baseline) vs. bystander lysis (indirect effect)

3.5.3 Mechanism Hypothesis

Selectivity arises from biophysical differences:

- **Tumor cells:** lower membrane tension, higher stiffness, distinct morphology
- **Normal fibroblasts:** higher membrane compliance, different mechanical properties

- Needles preferentially disrupt stiffer, morphologically distinct tumor cells

To test this, measure cell mechanical properties via AFM before lysis:

- Indentation modulus (stiffness) for cancer vs. normal cells ($n = 20$ cells each)
- Compare to lysis selectivity data

3.5.4 Acceptance Criteria

- Selectivity Index (cancer:normal): ≥ 3 (minimum); target ≥ 4
- Bystander fibroblast lysis: $\leq 20\%$ in co-culture condition
- Mechanical property differences: statistically significant ($p < 0.05$) between cancer and normal

3.5.5 Deliverable

Technical Report: “Selectivity Assessment: Cancer vs. Normal Cell Lysis”

3.6 Task 2.5: Cytokine Profiling & Tumor Lysis Syndrome Prevention (Weeks 10–13)

3.6.1 Objective

Measure systemic cytokine elevation post-lysis and assess whether chitosan sequestration prevents TLS marker accumulation.

3.6.2 Biomarkers (TLS Risk Markers)

- **Inflammatory cytokines:** IL-6, TNF- α , IL-1 β , IL-8 (indicate systemic inflammation risk)
- **TLS biochemical markers:**
 - Potassium (K^+): target ≤ 5.5 mM (normal: 3.5–5.0 mM)
 - Uric acid: target ≤ 8 mg/dL (normal: 3.5–7.2 mg/dL)
 - Phosphate (PO_4^{3-}): target ≤ 4.5 mg/dL (normal: 2.5–4.5 mg/dL)
 - Calcium (Ca^{2+}): monitor for secondary hypocalcemia

3.6.3 Experimental Groups

1. **Group A (Baseline):** Tumor spheroid in device, NO lysis (positive control for spheroid-derived cytokines)
2. **Group B (Lysis, No Chitosan):** Tumor spheroid + mechanical lysis, Hempoxy needles without chitosan coating
3. **Group C (Lysis + Chitosan):** Tumor spheroid + mechanical lysis, Hempoxy needles with chitosan coating (from Phase I)
4. **Group D (Chemical Lysis Control):** Tumor spheroid + detergent-induced lysis (1% Triton-X100, 5 min) as maximal inflammatory control

3.6.4 Protocol

1. Each group: $n = 3$ biological replicates (separate tumor spheroids)
2. Collect supernatant at timepoints: 0 (baseline), 5 min, 30 min, 2 hr, 6 hr post-lysis
3. Quantify biomarkers via:
 - Cytokines: Luminex multiplexed immunoassay or ELISA (IL-6, TNF- α , IL-1 β , IL-8)

- K^+ , PO_4^{3-} , Ca^{2+} : atomic absorption spectroscopy or ISE (ion-selective electrode)
 - Uric acid: enzymatic assay (uricase-catalyzed oxidation, colorimetric detection)
4. Statistical analysis: ANOVA + post-hoc Tukey test (Groups A vs. B vs. C vs. D)

3.6.5 Acceptance Criteria

- Group C (Lysis + Chitosan) IL-6 elevation: ≤ 5 pg/mL (vs. baseline)
- Group C K^+ elevation: ≤ 1 mM above baseline
- Group C shows statistically significant reduction in all biomarkers vs. Group B ($p < 0.05$)
- Group C comparable to or better than Group A (no lysis baseline)

3.6.6 Deliverable

Technical Report: “Cytokine & TLS Biomarker Analysis”

- Time-course plots for all 4 groups (cytokines + biochemical markers)
- Summary table with statistical analysis (ANOVA results, p -values)
- Assessment of chitosan efficacy in debris sequestration

3.7 Phase II Summary

Upon completion of Phase II (Months 4–6):

1. Tumor spheroid lysis efficiency: $\geq 90\%$ — **GATE CRITERION**
2. Selectivity (cancer:normal): ≥ 3 , target ≥ 4 — **GATE CRITERION**
3. Systemic cytokine elevation: ≤ 5 pg/mL IL-6 (with chitosan) — **GATE CRITERION**
4. EIS signatures validated; real-time monitoring method established

GO/NO-GO DECISION: If all criteria met, proceed to Phase III (AI/ML optimization). If selectivity < 3 or cytokines > 5 pg/mL, iterate Task 2.1–2.5 (optimize needle geometry, frequency, chitosan formulation) or reconsider platform approach.

4 PHASE III: AI TARGETING & OPTIMIZATION (Months 7–12)

4.1 Objectives

1. Develop machine learning (ML) model for real-time cancer vs. normal cell classification
2. Integrate ML with needle array control for targeted lysis
3. Achieve $\geq 95\%$ classification accuracy on held-out cell population
4. Validate targeting error $< 5\%$ (false-positive lysis of normal cells)

4.2 Task 3.1: Feature Engineering & Data Acquisition (Weeks 13–16)

4.2.1 Data Sources

Feature Vector: Electrical Impedance Spectroscopy (EIS) Signatures

Record impedance $Z(\omega)$ and phase $\theta(\omega)$ for 100 Hz–1 MHz frequency sweep.

1. Cell Lines (Training Data):

- Cancer: HeLa (cervical), MCF-7 (breast), A549 (lung) — $n = 200$ cells each
- Normal: human diploid fibroblasts (MRC-5 or equivalent), primary keratinocytes — $n = 200$ cells each
- Total: 800–1000 single cells, 2D culture in DMEM

2. 3D Spheroid Data (Validation):

- Tumor spheroids (mixed cell types from Phase II) — $n = 30$
- Normal fibroblast spheroids — $n = 30$
- EIS measured at spheroid surface via needle array

4.2.2 Impedance Measurement Protocol

1. AC voltage sweep: 100 Hz–1 MHz, 0.1 V amplitude
2. Measure at room temperature (20–22 °C) or 37 °C (physiologic)
3. Number of frequency points: 30–50 logarithmically spaced
4. Temporal resolution: measure every 30 sec for 10 min (cells settling/adhering)
5. Use interdigitated or parallel-plate electrode configuration (fixed geometry)

4.2.3 Feature Extraction

From raw impedance data $Z(f)$ and phase $\theta(f)$, compute features:

Frequency-Domain Features:

- Peak impedance magnitude: $Z_{\max}$
- Frequency at peak: $f_{\max}$
- Impedance at 1 kHz, 100 kHz, 1 MHz: Z_{1k}, Z_{100k}, Z_{1M}
- Phase angle at characteristic frequencies: $\theta_{1k}, \theta_{100k}, \theta_{1M}$
- Impedance slope (dZ/df): rough estimate of membrane permeability

Cole-Cole Model Fit Parameters:

- Fit impedance data to Cole-Cole dispersion: $Z^*(\omega) = Z_{\infty} + \frac{Z_0 - Z_{\infty}}{1 + (i\omega\tau)^{1-\alpha}}$
 - Z_0, Z_{∞} = low and high-frequency limits
 - τ = relaxation time constant
 - α = distribution parameter (ideality)
- Extract: $\tau, \alpha, \Delta Z = Z_0 - Z_{\infty}$ (membrane capacitance proxy)

Morphological Features (Microscopy Correlation):

- Cell diameter: d (optical microscopy)
- Circularity: $C = 4\pi A/P^2$ (where A = area, P = perimeter)
- Aspect ratio: long axis / short axis

Total feature vector: $\sim 15 - 20$ features per cell.

4.2.4 Deliverable

Dataset: “Impedance Signatures for Cancer vs. Normal Cells”

- Feature matrix: 800–1000 cells $\times$ 15–20 features
- Labels: binary (cancer = 1, normal = 0)
- Metadata: cell line, passage, measurement date/time
- Impedance curves (plot samples)

4.3 Task 3.2: Machine Learning Model Development (Weeks 16–20)

4.3.1 Approach

Train multiple classifier architectures and compare:

1. Logistic Regression

- Baseline linear classifier
- Interpretability: feature importance coefficients

2. Random Forest

- Ensemble decision trees; handles non-linear relationships
- Feature importance ranking

3. Support Vector Machine (SVM)

- RBF kernel (non-linear separation)
- Hyperparameter: C (regularization), γ (kernel width)

4. Neural Network

- 2–3 hidden layers (64–128 nodes each)
- Activation: ReLU; output: sigmoid (binary classification)

4.3.2 Training Procedure

1. Data Split:

- Training: 70% of 2D single-cell data ($n \sim 560$ cells)
- Validation: 15% ($n \sim 120$ cells) — hyperparameter tuning
- Test: 15% ($n \sim 120$ cells) — held-out performance estimate
- 3D spheroid data: reserved for independent validation

2. Preprocessing:

- Standardization: zero mean, unit variance (Z-score normalization)
- Handle missing values: imputation (mean or forward-fill)
- Feature scaling: necessary for SVM and neural networks

3. Cross-Validation:

- 5-fold stratified cross-validation on training set
- Metric: Area Under Receiver Operating Characteristic curve (AUROC)
- Monitor: sensitivity (true positive rate), specificity (true negative rate)

4. Hyperparameter Optimization:

- Grid search or Bayesian optimization on validation set
- Optimize for: AUROC or F1-score (balance precision–recall)

4.3.3 Model Evaluation Metrics

- **Accuracy:** $\frac{TP+TN}{TP+TN+FP+FN}$
- **Sensitivity (Recall):** $\frac{TP}{TP+FN}$ (true positive rate; ability to identify cancer cells)
- **Specificity:** $\frac{TN}{TN+FP}$ (true negative rate; ability to identify normal cells)
- **Precision:** $\frac{TP}{TP+FP}$ (among predicted cancer, what fraction is truly cancer)
- **F1-Score:** $2 \times \frac{\text{Precision} \times \text{Recall}}{\text{Precision} + \text{Recall}}$
- **AUROC:** area under ROC curve (trade-off sensitivity vs. specificity)

4.3.4 Acceptance Criteria

- Test set accuracy: $\geq 95\%$ — **GATE CRITERION**
- Sensitivity: $\geq 95\%$ (minimize false negatives; must catch cancer cells)
- Specificity: $\geq 95\%$ (minimize false positives; avoid lysing normal cells)
- AUROC: ≥ 0.99 (near-perfect discrimination)

4.3.5 Deliverable

Technical Report: “ML Model Development & Validation”

- ROC curves (all classifier types)
- Confusion matrices (test set)
- Feature importance ranking (Random Forest + SVM)
- Cross-validation results (mean $\pm$ SD across 5 folds)

4.4 Task 3.3: Real-Time Targeting Algorithm & Integration (Weeks 20–24)

4.4.1 Objective

Implement ML model on microcontroller and integrate with needle array actuator for live decision-making.

4.4.2 Hardware Architecture

1. EIS Measurement Unit:

- Potentiostat: Analog Devices AD5933 or equivalent (integrate on PCB)
- Frequency sweep: 100 Hz–1 MHz in software
- Sampling rate: 1 kHz (sufficient for impedance measurement)
- Measurement cycle time: ~ 1 second per single-point EIS

2. Microcontroller:

- ARM Cortex-M7 (e.g., STM32H7 series) or equivalent
- Onboard 256 KB RAM, 512 KB flash (adequate for ML model)
- Real-time OS: FreeRTOS for task scheduling

3. Needle Array Driver:

- Voltage amplifier (PZT drive: 0–150 V, programmable)
- Multiplexer: 100 independently addressable needles
- Frequency generator: 10–200 Hz, programmable waveforms (sine, square, triangle)

4.4.3 Software Algorithm

Real-Time Targeting Logic (Pseudocode):

LOOP (every 100 ms):

FOR each of 100x100 needle positions:

1. Measure EIS at current position (1 sec per measurement)
2. Extract impedance features (z_{1k} , z_{100k} , $phase_{1k}$, etc.)
3. Normalize features (Z-score using training set statistics)
4. Feed into ML classifier (trained model loaded in memory)
5. IF classifier output > 0.5 THEN:

```

        likelihood = cancer cell HIGH
        Activate needle (100--200 Hz, 2--5 sec)
        Wait for debris sequestration (chitosan binding, ~5 sec)
    ELSE:
        likelihood = normal cell LIKELY
        Skip needle activation
6. Update impedance map (spatial distribution of cancer vs. normal)
END FOR
OUTPUT: lysis efficiency map, coverage statistics
END LOOP

```

4.4.4 Rolling-Wave Activation Pattern

To localize debris release and enable chitosan sequestration:

- Divide 100×100 array into 10×10 subgrids (100 subgrids total)
- Activate each subgrid sequentially with 100 ms delay between subgrids
- Rationale: debris from subgrid n diffuses and binds to chitosan before subgrid $n + 1$ is activated
- Total activation time: $100 \times 0.1 = 10$ seconds per full-array pass

4.4.5 Acceptance Criteria

- Measurement latency: ≤ 2 seconds per needle location (EIS + classification)
- Model inference time: ≤ 10 ms per cell (on-device computation)
- Targeting accuracy: $\geq 95\%$ agreement with test set predictions
- False positive rate (normal cell activation): $\leq 5\%$

4.4.6 Deliverable

Technical Documentation: “AI Targeting Algorithm & Hardware Integration”

- Circuit schematics (EIS, amplifier, multiplexer, microcontroller)
- Firmware flowchart (measurement, classification, activation logic)
- Latency/throughput analysis
- Integration test results

4.5 Task 3.4: Validation on 3D Spheroid Co-Cultures (Weeks 21–24)

4.5.1 Objective

Validate ML-guided targeting on realistic 3D mixed spheroids (cancer + normal cells).

4.5.2 Experimental Design

1. Spheroid Types:

- Mono-culture: 100% cancer cells ($n = 10$ spheroids)
- Mono-culture: 100% normal fibroblasts ($n = 10$ spheroids)
- Co-culture: 50% cancer + 50% normal ($n = 10$ spheroids) — realistic heterogeneous tumor

2. Protocol:

- Embed spheroids in tumor-on-chip device (from Phase II)
- Run ML-guided targeting algorithm (real-time EIS + classification + needle activation)
- Record impedance maps and needle activation patterns
- Measure lysis efficiency via live/dead stain (post-lysis, 1 hr timepoint)
- Quantify selectivity in co-cultures: cancer lysis vs. fibroblast lysis

4.5.3 Metrics

• Lysis Efficiency (Mono-Cultures):

- Cancer: target $\geq 90\%$
- Normal: target $\leq 10\%$ (should NOT be lysed)

• Selectivity (Co-Cultures):

- Cancer cells lysed: target $\geq 85\%$
- Normal cells lysed: target $\leq 15\%$
- Selectivity Index: (cancer lysis) / (normal lysis) ≥ 4

• Targeting Accuracy:

- Compare impedance-based predictions to post-lysis cell identification
- Confusion matrix: how many cells were correctly classified before lysis?
- Accuracy: $\geq 95\%$ (matches ML test set performance)

4.5.4 Deliverable

Technical Report: “AI-Guided Targeting Validation on 3D Spheroids”

- Impedance maps (spatial distribution, pre- and post-lysis)
- Live/dead microscopy images
- Lysis efficiency & selectivity summary table
- Confusion matrix (predicted vs. actual cell type)

4.6 Phase III Summary

Upon completion of Phase III (Months 7–12):

1. ML model test set accuracy: $\geq 95\%$ (sensitivity + specificity) — **GATE CRITERION**
2. Real-time targeting on 3D co-cultures: selectivity ≥ 4 (cancer:normal) — **GATE CRITERION**
3. Hardware integration complete; real-time measurement latency ≤ 2 sec per location
4. AI algorithm documentation and firmware finalized

GO/NO-GO DECISION: If all criteria met, proceed to Phase IV (preclinical efficacy in vivo). If accuracy $< 95\%$ or selectivity < 3 , iterate Task 3.1–3.4 or consider alternative ML architectures.

5 PHASE IV: PRECLINICAL EFFICACY IN VIVO (Months 13–18)

5.1 Objectives

1. Demonstrate tumor regression in mouse xenograft models
2. Compare efficacy to standard chemotherapy + TLS prophylaxis
3. Assess systemic safety (histopathology, organ toxicity)
4. Validate TLS prevention (serum K^+ , urate, phosphate)

5.2 Task 4.1: Xenograft Tumor Model Development (Weeks 25–30)

5.2.1 Model Selection

Subcutaneous Xenograft (Primary Model):

- Host: immunocompromised mice (NSG — NOD/SCID/IL-2R γ null, or equivalent)
- Tumor cells: HeLa or MCF-7 (established), or patient-derived xenograft (PDX)
- Implantation: 2×10^6 cells in 50 μ L PBS, subcutaneous flank injection
- Growth: allow 2–3 weeks until tumor reaches 150–300 mm³ (measurable, before exponential growth)
- Number of mice: $n = 30$ total (subdivided into groups, see Task 4.2)

5.2.2 Tumor Monitoring

- Calipers: measure length (L) and width (W); volume $V = 0.5 \times L \times W^2$ (mm³)
- Frequency: 2–3 times per week
- Target baseline: all groups achieve ~ 200 mm³ before treatment randomization

5.2.3 Approval & Regulatory Compliance

- IACUC protocol approval (institutional Animal Care & Use Committee)
- Ethical endpoint: tumor burden ≤ 1000 mm³ or sign of distress (weight loss $> 15\%$)
- Humane euthanasia: CO₂ inhalation followed by cervical dislocation

5.3 Task 4.2: Treatment Randomization & Efficacy Study (Weeks 30–42)

5.3.1 Study Design

Randomized Controlled Trial (RCT):

Group	Treatment	N
A (Control)	Vehicle (PBS) intravenous	5
B (Chemo Only)	Doxorubicin 5 mg/kg i.v., single dose	8
C (Chemo + TLS Prophylaxis)	Doxo + allopurinol (100 mg/kg p.o., daily)	8
D (Hempoxy Mech. Lysis)	Needle array implant + AI-guided activation, weekly $\times$ 3	6
E (Hempoxy + Chemo)	Combination: Doxo + needle array activation	3

5.3.2 Treatment Protocol

Group B & C: Doxorubicin (Chemotherapy)

- Dose: 5 mg/kg intravenous (via tail vein)
- Single administration on Day 0
- Doxorubicin is a broad-spectrum DNA intercalator; induces rapid tumor cell death
- Expected TLS risk: moderate-to-high (particularly in rapidly growing tumors)

Group C: Allopurinol (TLS Prophylaxis)

- Dose: 100 mg/kg per day (dissolved in drinking water or oral gavage)
- Start: Day -3 (3 days pre-chemotherapy) through Day 7 post-chemotherapy
- Mechanism: xanthine oxidase inhibitor; reduces uric acid production

Group D & E: Hempoxy Mechanical Lysis

- Surgical implantation: place needle array device in subcutaneous pocket adjacent to tumor (under general anesthesia + analgesics)
- Activation schedule: weekly starting Day 1; total 3 sessions (Days 1, 8, 15)
- Each session: 30 min continuous AI-guided targeting (EIS + classification + rolling-wave activation)
- Frequency: 150 Hz, amplitude optimized from Phase II
- Post-operative care: analgesia (buprenorphine 0.1 mg/kg q8h $\times$ 3 days)

5.3.3 Primary Endpoint

Tumor Volume Reduction: Percent change in tumor volume from baseline to Day 21 (end of treatment window).

- Hypothesis: Group D (mechanical lysis) and Group E (combined) show similar or superior tumor reduction vs. Group B (chemo only)

5.3.4 Secondary Endpoints

- **Time to progression:** days from baseline to $2\times$ baseline tumor volume
- **Overall survival:** days from treatment start to euthanasia
- **Body weight:** monitor for toxicity (expected: stable in Groups A, D; weight loss in Groups B, C if severe TLS)
- **TLS biomarkers:** serum K^+ , urate, phosphate, Ca^{2+} (see Task 4.3)

5.4 Task 4.3: TLS Biomarker & Safety Monitoring (Throughout Weeks 30–42)

5.4.1 Blood Sampling

- Timepoints: Days 0 (baseline), 1, 3, 7, 14, 21
- Volume: 50–100 μL per draw (via retro-orbital venipuncture or saphenous vein)
- Serum preparation: centrifuge 10 min, separate, store at $-80\text{ }^{\circ}\text{C}$

5.4.2 Biomarker Analysis

- **TLS Markers:**
 - Potassium (K^+): inductively coupled plasma mass spectrometry (ICP-MS) or flame photometry (normal mouse: 3.5–5.0 mM)
 - Uric acid: HPLC or enzymatic assay (normal: $\sim 0.3 - 0.5\text{ mg/dL}$ in mice; TLS risk if $> 5\text{ mg/dL}$)
 - Phosphate (PO_4^{3-}): ICP-MS or colorimetric assay (normal: 4–8 mg/dL in mice)
 - Calcium (Ca^{2+}): flame photometry or ICP-MS (normal: 8–10 mg/dL; monitor for secondary hypocalcemia)
- **Organ Dysfunction Markers:**
 - Creatinine: enzymatic assay (renal function proxy; normal mouse: 0.3–0.6 mg/dL)
 - Blood urea nitrogen (BUN): urease-based assay (normal: 15–30 mg/dL)
 - Alanine aminotransferase (ALT), aspartate aminotransferase (AST): spectrophotometric assay (hepatic markers)
- **Inflammatory Markers:**
 - Cytokines: IL-6, TNF- α , IL-1 β (Luminex or ELISA; normal mouse: $< 5\text{ pg/mL}$ baseline)

5.4.3 Statistical Analysis

- Two-way ANOVA: treatment group $\times$ timepoint
- Post-hoc: Tukey’s HSD test for pairwise comparisons (Groups A, B, C, D, E)
- Outcome: which groups show statistically different biomarker trajectories?
- Target: Group D shows biomarker profile similar to Group A (no treatment) despite tumor lysis

5.5 Task 4.4: Histopathology & Organ Toxicity Assessment (Week 42)

5.5.1 Terminal Necropsy

At study endpoint (Day 21 or ethical endpoint if earlier), euthanize all mice and collect tissues:

- **Tumor:** formalin-fixed, paraffin-embedded (FFPE) for histology
- **Organs:** heart, liver, kidney, spleen (FFPE)
- **Needle implant site:** tissue surrounding device (assess inflammation, foreign body response)

5.5.2 Histological Analysis

1. Hematoxylin & Eosin (H&E) staining (standard)

- Assess: tumor necrosis, cell infiltration, tissue architecture
- Grading: % necrotic area in treated vs. control tumors

2. Immunohistochemistry (IHC)

- Apoptosis marker: active caspase-3 or TUNEL stain (quantify % apoptotic cells)
- Proliferation: Ki-67 (quantify % cycling cells; expect lower in treated groups)
- Immune infiltration: CD8⁺ T cells (indicate anti-tumor immune response)

3. Organ toxicity assessment (qualitative)

- Liver, kidney, heart: look for necrosis, infiltration, degeneration
- Needle implant site: inflammatory infiltrate, fibrosis, tissue necrosis
- Quantify: inflammation severity score (0 = none, 1 = minimal, 2 = moderate, 3 = severe)

5.5.3 Acceptance Criteria

- **Tumor response:** Group D shows tumor volume reduction $\geq 30\%$ from baseline (or similar to Group B/C)
- **Necrosis:** Group D tumors show $\geq 50\%$ necrotic area (evidence of cell death)
- **Organ toxicity:** liver/kidney inflammation score ≤ 1 in Group D (acceptable safety)
- **Device tolerability:** needle implant site inflammation ≤ 2 (acceptable foreign body response)

5.5.4 Deliverable

Technical Report: “Preclinical Efficacy & Safety Assessment”

- Tumor growth curves (all groups, mean $\pm$ SEM)
- TLS biomarker time-courses (all groups)
- Histology images with quantification (necrosis %, apoptosis %, immune infiltration)
- Organ toxicity grading summary
- Statistical analysis (ANOVA, p -values, post-hoc tests)

5.6 Phase IV Summary

Upon completion of Phase IV (Months 13–18):

1. Tumor regression: Group D $\geq$ 30% volume reduction (or non-inferior to chemotherapy)
2. TLS prevention: Group D biomarkers (K^+ , urate) remain stable (superior to Group B)
3. Safety: acceptable organ toxicity and device tolerability
4. Data sufficient for IND application (regulatory pathway to human trials)

SUCCESS OUTCOME: Platform demonstrates efficacy and improved safety profile (reduced TLS) compared to chemotherapy alone.

6 PUBLICATION STRATEGY

6.1 Priority 1: Materials Science (Months 1–3, publish Month 6)

Journal Target: *Advanced Materials*, *Biomaterials*, or *Composites Science and Technology*

Manuscript: “Hempoxy Biocomposite Tensile Characterization and Nanoparticle Reinforcement for Medical Microneedles”

- Focus: material property validation, nanoparticle optimization, structure–property relationships
- Scope: Phase I data only (tensile testing, nanoindentation, SEM fractography)
- Novelty: first application of hemp/epoxy composites to microneedle arrays (to authors’ knowledge)

6.2 Priority 2: Bioengineering (Months 4–6, publish Month 10)

Journal Target: *Advanced Biomedical Engineering*, *Lab on a Chip*, or *ACS Biomaterials Science & Engineering*

Manuscript: “pH-Triggered Chitosan Debris Sequestration for Mechanical Tumor Cell Lysis: In Vitro Kinetics and Efficacy in Tumor-on-Chip Models”

- Focus: chitosan binding mechanism, 3D spheroid lysis, selectivity assessment
- Scope: Phase II data (microneedle fabrication, EIS characterization, lysis assays, cytokine profiling)
- Novelty: first demonstration of localized intracellular debris sequestration at point-of-lysis

6.3 Priority 3: AI/Biomedical (Months 7–12, publish Month 16)

Journal Target: *IEEE Transactions on Biomedical Engineering*, *Nature Biomedical Engineering*, or *Science Translational Medicine*

Manuscript: “Real-Time AI-Guided Mechanical Cell Disruption: Machine Learning Classification of Cancer vs. Normal Cells via Electrical Impedance Spectroscopy”

- Focus: ML model development, real-time targeting, feature engineering
- Scope: Phase III data (impedance signatures, classifier performance, 3D validation)
- Novelty: first integration of EIS-based AI targeting with mechanical lysis in tumor models

6.4 Priority 4: Oncology (Months 13–18, publish Month 22)

Journal Target: *Cancer Research*, *Molecular Cancer Therapeutics*, or *Nature Cancer*

Manuscript: “Mechanical Tumor Disruption with AI-Guided Hempoxy Microneedles: Preclinical Efficacy and Prevention of Tumor Lysis Syndrome in Xenograft Models”

- Focus: in vivo efficacy, TLS prevention, comparison to standard chemotherapy
- Scope: Phase IV data (tumor growth, biomarkers, histopathology, safety)
- Novelty: first mechanical oncology approach with integrated TLS prevention
- Clinical significance: reduced systemic burden of TLS prophylaxis

6.5 Ancillary Publications

Patent Disclosure Manuscripts: If IP strategy warrants, publish preliminary findings before patent filing (to establish priority date). Consider:

- Brief technical note on chitosan coating method (Phase II)
- Methods paper on EIS-based impedance feature extraction (Phase III)

Perspective/Review: After all phases complete, co-author perspective on “Mechanical Oncology: Physics-Based Alternatives to Chemotherapy.”

7 INTELLECTUAL PROPERTY & PATENT STRATEGY

7.1 Core IP Assets

1. Hemp-Based Composite Microneedles

- Claim: conical Hempoxy microneedle geometry with specified dimensions
- Scope: composition, fabrication method, mechanical properties
- Priority: file provisional patent before first publication (establish priority date)

2. pH-Triggered Chitosan Debris Sequestration Coating

- Claim: chitosan + genipin cross-linker; pH-sensitive binding mechanism
- Scope: coating formulation, intracellular debris binding (K^+ , nucleotides, phosphate)
- Embodiment: application to medical microneedles, oncology context

3. AI-Guided Mechanical Targeting Algorithm

- Claim: ML classifier trained on EIS impedance signatures
- Scope: real-time cancer vs. normal cell discrimination
- Implementation: firmware, hardware integration (microcontroller + potentiostat)
- Method claim: rolling-wave needle activation pattern

4. Integrated Tumor-on-Chip Device

- Claim: microfluidic 3D spheroid chamber with integrated needle array and EIS electrodes
- Scope: design, fabrication, operational use in preclinical testing

7.2 Patent Filing Strategy

Timeline:

- **Month 2 (Phase I, interim):** File provisional patent application covering hemp composite microneedles and chitosan coating mechanism
 - Rationale: establish priority date before publications
 - Disclosure: reference Phase I preliminary data

- **Month 9 (Phase II complete):** Convert provisional to PCT (Patent Cooperation Treaty) application
 - Expand claims to include tumor-on-chip integration and efficacy data
 - Maintain single-patent-family strategy (avoid divisional fragmentation)
- **Month 15 (Phase III complete):** File continuation or CIP (Continuation-in-Part) application
 - Add claims for AI targeting algorithm and ML implementation
 - New matter: impedance feature extraction, classifier architecture
- **Month 20 (Phase IV interim):** Enter national phases (US, Europe, Canada, Japan, Australia) via PCT
 - Strategic jurisdiction selection based on commercialization plan

Publication Coordination:

- Always file provisional patent BEFORE manuscript submission to journals
- Embargo journals for 12 months if necessary to maintain trade secret status (if not patented)
- Prioritize patent issuance over rapid publication to protect IP

7.3 Trade Secrets & Know-How

Document and protect:

- Exact Hempoxy formulation (fiber/resin ratio, nanoparticle concentrations, cure schedule)
- Optimal chitosan cross-linking parameters (genipin concentration, temperature, time)
- ML model training data and hyperparameter optimization results
- Microneedle fabrication mold specifications and demolding conditions

8 BUDGET FRAMEWORK

8.1 Phase I: Material Characterization (Months 1–3)

Budget Category	Unit Cost	Total (3 mo)
Materials & Supplies		
Hemp fiber (10 kg)	\$50/kg	\$500
Epoxy resin (25 L)	\$80/L	\$2,000
Graphene oxide nanosheets (10 g)	\$200/g	\$2,000
Boron nitride nanoplatelets (10 g)	\$150/g	\$1,500
Chitosan (100 g)	\$30/g	\$3,000
Genipin (5 g)	\$200/g	\$1,000
Miscellaneous (solvents, couplers)		\$1,000
Equipment (amortized 3 mo)		
Instron tensile tester	\$60,000 (capital)	\$5,000
Scanning electron microscope (SEM)	\$200,000 (capital)	\$5,000
Atomic force microscope (AFM)	\$300,000 (capital)	\$7,500
Scintillation counter (radiometric)	\$80,000 (capital)	\$2,000
Furnace/ovens (multiple)	\$5,000 (capital)	\$400
Personnel		
Postdoctoral researcher (0.5 FTE)	\$65,000/yr	\$16,250
Graduate student (1.0 FTE)	\$35,000/yr	\$8,750
Technician (0.5 FTE)	\$40,000/yr	\$5,000
PI time (0.1 FTE)	\$150,000/yr	\$3,750
Subcontracts		
Tensile testing (commercial lab)	\$500/specimen	\$2,500
Subtotal Phase I		\$67,650

8.2 Phase II: Tumor-on-Chip (Months 4–6)

Budget Category	Unit Cost	Total (3 mo)
Materials & Supplies		
PDMS (Dow Corning) (500 g)	\$100/100 g	\$500
Cell culture media (DMEM, FBS)		\$2,000
Matrigel/collagen matrices	\$200/mL	\$1,000
Primary tumor cells/lines		\$3,000
Cancer cell lines (HeLa, MCF-7, A549)		\$2,000
Normal fibroblasts (MRC-5)		\$1,000
Live/dead staining kits		\$1,000
Cytokine ELISA/Luminex kits	\$150/assay	\$2,250
Ion chromatography standards		\$1,000
Miscellaneous (plasticware, tips)		\$2,000
Equipment (amortized)		
Microfluidic pump system	\$15,000 (capital)	\$1,250
Inverted microscope + imaging	\$50,000 (capital)	\$4,167
Incubator (CO ₂ -controlled)	\$8,000 (capital)	\$667
Potentiostat/EIS instrument	\$35,000 (capital)	\$2,917
Personnel		
Postdoctoral researcher (1.0 FTE)	\$65,000/yr	\$16,250
Graduate student (1.0 FTE)	\$35,000/yr	\$8,750
Technician (1.0 FTE)	\$40,000/yr	\$10,000
Subcontracts		
Cytokine multiplex assay (commercial)	\$500/sample	\$3,000
Subtotal Phase II		\$62,802

8.3 Phase III: AI/ML (Months 7–12)

Budget Category	Unit Cost	Total (6 mo)
Materials & Supplies		
Cell culture (continued)		\$3,000
Electrochemistry supplies		\$1,500
PCB fabrication (10 iterations)	\$500/batch	\$5,000
Microcontroller/firmware development		\$2,000
Equipment (amortized)		
(Potentiostat, microscope from Phase II)		\$3,500
Workstations (2 × laptops for ML)	\$2,000 each	\$4,000
Software		
MATLAB/Python licenses (ongoing)	\$500/yr	\$250
ML libraries (TensorFlow, scikit-learn)	Open-source	\$0
Personnel		
Computer scientist/ML engineer (1.0 FTE)	\$90,000/yr	\$45,000
Postdoctoral researcher (0.5 FTE)	\$65,000/yr	\$16,250
Graduate student (1.0 FTE)	\$35,000/yr	\$17,500
Subcontracts		
PCB design/fabrication consulting	\$150/hr × 100 hr	\$15,000
Subtotal Phase III		\$113,000

8.4 Phase IV: Preclinical Efficacy (Months 13–18)

Budget Category	Unit Cost	Total (6 mo)
Animal Care & Supplies		
NSG mice (30 mice @ \$150 each)	\$150/mouse	\$4,500
Mouse housing/care (6 months)	\$50/mouse/month	\$9,000
Doxorubicin (chemotherapy)	\$500 (5 doses)	\$500
Allopurinol	\$100/batch	\$200
Surgical & Monitoring		
Surgical supplies (anesthesia, sutures)		\$2,000
Ultrasound imaging (tumor monitoring)		\$3,000
Blood chemistry analysis (ICP-MS, assays)	\$200/sample $\times$ 200 samples	\$40,000
Histopathology & IHC		
Tissue processing (FFPE blocks)	\$50/sample $\times$ 30 samples	\$1,500
H&E staining	\$20/slide $\times$ 60 slides	\$1,200
IHC staining (3 markers)	\$200/marker $\times$ 3 $\times$ 30 tissues	\$18,000
Pathology consultation	\$150/hr $\times$ 20 hr	\$3,000
Personnel		
Veterinary technician (1.0 FTE)	\$45,000/yr	\$22,500
Postdoctoral researcher (1.0 FTE)	\$65,000/yr	\$32,500
Graduate student (1.0 FTE)	\$35,000/yr	\$17,500
Subcontracts		
Commercial pathology lab	per slide	\$5,000
Subtotal Phase IV		\$160,900

8.5 TOTAL 18-MONTH BUDGET

Phase	Cost
I (Material Characterization)	\$67,650
II (Tumor-on-Chip)	\$62,802
III (AI/ML Targeting)	\$113,000
IV (Preclinical Efficacy)	\$160,900
SUBTOTAL (Direct)	\$404,352
Indirect (Overhead @ 25%)	\$101,088
TOTAL PROJECT COST	\$505,440

Cost per month: $\approx$ \$28,000 average (variable by phase)

Major capital equipment purchases (one-time): $\sim$ \$700,000 (amortized separately if not yet in place at institution)

9 RISK MITIGATION & CONTINGENCY PLANNING

9.1 Identified Risks

Risk	Likelihood	Impact	Mitigation
Hempoxy < 100 MPa	Medium	High	Iterate nanoparticle content in Task 1.1; or switch to alternative fiber (Kevlar)
Chitosan binding < 70%	Medium	High	Test alternative coatings: alginate, hyaluronic acid, or optimize genipin dose
Selectivity < 3	Medium	High	Investigate biophysical differences (AFM); redesign needle geometry or frequency
ML accuracy < 95%	Low–Medium	Medium	Collect more training data (Task 3.1); try ensemble methods or deep learning
Tumor model fails	Low	Very High	Maintain backup PDX lines; test in orthotopic model if flank xenografts unsuccessful
TLS markers elevated	Low–Medium	Medium	Increase chitosan coating density or test combination with allopurinol (Group E)
Device implant rejection	Low	Medium	Optimize PEG coating; test biocompatible encapsulation
Regulatory delay	Low	Medium	Engage FDA early (IND-enabling studies); consider accelerated pathway (breakthrough therapy)

9.2 Contingency Actions

1. If Phase I fails (material strength):

- Pause Phases II–IV
- Increase nanoparticle content (2 wt% → 5 wt%) or try alternative reinforcement (carbon nanotubes, despite sustainability concerns)
- Alternatively: pivot to different needle material (polycarbonate, polystyrene, but loses biocompatibility advantage)

- Timeline impact: +2–4 weeks

2. If Phase II selectivity < 3:

- Do NOT advance to Phase III/IV
- Conduct supplementary biophysics study: AFM indentation on cancer vs. normal cells
- Redesign needle geometry (sharper tips, different taper angle)
- Test alternative activation frequencies (50 Hz, 100 Hz, 200 Hz)
- Timeline impact: +6–8 weeks

3. If Phase III ML accuracy < 90% on test set:

- Do NOT implant devices in mice
- Revisit feature engineering: try alternative impedance models (Cole-Cole vs. Debye)
- Collect additional training data from new cell lines
- Consider ensemble classifier or deep neural network
- Timeline impact: +4–6 weeks

4. If Phase IV tumor model fails:

- Initiate backup orthotopic (intramammary for breast, lung for lung) xenograft
- Coordinate with commercial CRO (contract research organization) if internal animal facility unavailable
- Extend Phase IV timeline to +8–12 weeks

10 REGULATORY PATHWAY

10.1 IND Application (Investigational New Drug) — FDA

10.1.1 Eligibility Criteria for Filing

- Phase I–IV preclinical data demonstrating safety and efficacy in animal models
- Manufacturing, chemistry, controls (CMC) section: standardized Hempoxy composite formulation, needle array quality control
- Pharmacology and toxicology: comprehensive biocompatibility and organ toxicity assessment

10.1.2 Timeline

- **Month 20:** Compile comprehensive IND package (preclinical data, manufacturing specs, regulatory history)
- **Month 22:** Submit IND application to FDA
- **Month 24:** FDA 30-day review period; expect feedback or approval
- **Month 25+:** If approved, initiate Phase I human trial (safety, dosage escalation in small patient cohort)

10.2 Breakthrough Therapy Designation (BTD)

Request BTD from FDA if preliminary Phase IV data show:

- Substantial improvement over standard-of-care (e.g., tumor regression + TLS prevention)
- Potential for serious or life-threatening TLS complications

BTD accelerates review timelines and facilitates access for eligible patients.

11 SUCCESS CRITERIA & GO/NO-GO GATES

11.1 Phase I Gate (Month 3 End-of-Phase Meeting)

All criteria must be MET to proceed to Phase II:

1. Hempoxy tensile strength ≥ 100 MPa (nanoparticle-reinforced) ✓
2. Microneedle piercing force 50–100 nN ✓
3. Microneedle buckling force > 500 nN ✓
4. Chitosan binding efficiency (K^+) $\geq 70\%$ at $t_{90} \leq 5$ sec ✓
5. Biocompatibility acceptable (hemolysis $\leq 5\%$; cytotoxicity $\leq 20\%$) ✓

If any criterion fails: Return to Task 1.1–1.4 or reassess platform feasibility.

11.2 Phase II Gate (Month 6 End-of-Phase Meeting)

All criteria must be MET to proceed to Phase III:

1. Tumor spheroid lysis $\geq 90\%$ (by live/dead stain) ✓
2. Selectivity index (cancer:normal) ≥ 3 (minimum); target ≥ 4 ✓
3. IL-6 elevation ≤ 5 pg/mL with chitosan sequestration ✓
4. EIS signatures validated; real-time monitoring demonstrated ✓

If selectivity < 3 or cytokines > 10 pg/mL: Iterate Tasks 2.1–2.5 or reconsider approach.

11.3 Phase III Gate (Month 12 End-of-Phase Meeting)

All criteria must be MET to proceed to Phase IV:

1. ML model test set accuracy $\geq 95\%$ (sensitivity + specificity both $\geq 95\%$) ✓
2. AI-guided targeting on 3D spheroids: selectivity ≥ 4 (cancer:normal) ✓
3. Hardware integration complete; measurement latency ≤ 2 sec/location ✓
4. Algorithm firmware finalized and validated ✓

If accuracy $< 90\%$: Revisit feature engineering or try alternative ML architectures.

11.4 Phase IV Gate (Month 18 Final Report)

Success Criteria:

1. Tumor regression: Group D $\geq$ 30% volume reduction OR non-inferior to Group B (chemotherapy) ✓
2. TLS prevention: Group D biomarkers (K^+ , urate, phosphate) remain stable vs. Group B ✓
3. Organ toxicity acceptable (liver/kidney inflammation score $\leq$ 1) ✓
4. Device tolerability: needle implant site inflammation $\leq$ 2 (moderate) ✓
5. Sufficient data for IND regulatory pathway ✓

Outcome: If all criteria met, platform advances to human trials (IND application in Month 22).

12 MANAGEMENT & GOVERNANCE

12.1 Project Leadership

- **Principal Investigator:** Marie-Soleil Seshat Landry (CEO, Landry Industries)
- **Project Manager:** [Designate] — oversee timeline, budget, deliverables
- **Phase I Lead (Materials):** [PhD Materials Scientist or Chemical Engineer]
- **Phase II Lead (Biomedical):** [PhD Biomedical Engineer]
- **Phase III Lead (AI/ML):** [PhD Computer Scientist or ML Engineer]
- **Phase IV Lead (Preclinical):** [DVM or PhD with animal research experience]

12.2 Governance Structure

1. Steering Committee (Monthly meetings)

- Members: PI, Phase leads, Project Manager, external advisor (oncology or biomedical device expert)
- Agenda: milestone status, risk assessment, budget review, publication strategy

2. Technical Working Groups (Weekly)

- Phase I: materials characterization team
- Phase II: tumor-on-chip team
- Phase III: AI/ML team
- Phase IV: preclinical efficacy team

3. External Advisory Board (Quarterly)

- Members: 3–5 external experts in oncology, biomedical engineering, regulatory affairs
- Role: independent review, risk assessment, guidance on clinical pathway

12.3 Decision-Making Criteria

Go/No-Go Decisions: Evaluated at each phase-end milestone (Months 3, 6, 12, 18).

- **GO:** All success criteria met; proceed to next phase on schedule.

- **GO WITH MODIFICATIONS:** Minor criteria missed; proceed with additional targeted work in parallel (does not delay main timeline).
- **NO-GO:** Major criteria missed; pause, investigate, and replan.

13 CONCLUSION

The Hempoxy Mechanical Oncology Platform represents an integrated, multidisciplinary research program bridging materials science, bioengineering, artificial intelligence, and clinical oncology. This Master R&D Plan provides a structured 18-month pathway from material characterization (Phase I) through preclinical efficacy (Phase IV), with explicit success criteria, risk mitigation, publication strategy, and IP protection.

Key Innovation: Mechanical (rather than pharmacological) tumor cell disruption combined with localized, AI-guided debris sequestration to prevent tumor lysis syndrome.

Potential Impact: If successful, this platform could reduce the systemic burden of TLS prophylaxis in cancer patients and provide an alternative or complementary approach to chemotherapy.

Next Steps:

1. Secure funding (\$505,440 total direct costs over 18 months)
2. Assemble multidisciplinary team (materials scientists, biomedical engineers, ML engineers, veterinary researchers)
3. Establish collaborations with academic medical center (animal facility, histopathology core, regulatory affairs)
4. Initiate Phase I experiments by Month 1
5. Target IND filing by Month 22 and Phase I human trial initiation by Month 25

References

- [1] Dhaliwal, G. S., Dueck, S. M., & Newaz, G. M. (2019). “Experimental and numerical characterization of mechanical properties of hemp fiber reinforced composites using multiscale analysis approach.” *SN Applied Sciences*, 1, 1383.
- [2] Islam, M., et al. (2025). “Mechanical properties of hemp fiber-reinforced thermoset and thermoplastic polymer composites: A comprehensive review.” *SPE Polymers*, 6(1), 10173.
- [3] Erklig, A., et al. (2025). “Investigation of Mechanical and Impact Properties of Hemp Fiber Reinforced Epoxy-Based Nanocomposites.” *Polymer Composites*.
- [4] Chen, S. (2003). “Cells lying on a bed of microneedles: An approach to isolate mechanical force.” *Proceedings of the National Academy of Sciences USA*, 100(4), 1484–1489.
- [5] Wei, M., et al. (2014). “Poking cells for efficient vector-free intracellular delivery.” *Nature Communications*, 5, 4466.
- [6] (2021). “Cell penetration efficiency analysis of different atomic force microscopy nanoneedles into living cells.” *Scientific Reports*, 11, 8015.
- [7] Kim, J., Hong, J. W., Kim, D. P., Shin, J. H., & Park, I. (2012). “Nanowire-integrated microfluidic devices for facile and reagent-free mechanical cell lysis.” *Lab on a Chip*, 12, 2914–2921.
- [8] (2024). “Tumor Lysis Syndrome.” *StatPearls*, National Center for Biotechnology Information.
- [9] Perissinotti, A. J., et al. (2023). “Expert consensus guidelines for the prophylaxis and management of tumor lysis syndrome in the United States.” *Cancer Treatment Reviews*, 120, 102603.
- [10] Harris, J. M., & Chess, R. B. (2003). “Effect of pegylation on pharmaceuticals.” *Nature Reviews Drug Discovery*, 2(3), 214–221.