COVER PAGE

Official Title: Mn/QD-SAC–Guided NIR-IIb Imaging and Antioxidant Therapy for Ischemia–Reperfusion Injury in DIEP Flaps

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1. SYNOPSIS

（1）Background: Deep inferior epigastric perforator (DIEP) flap breast reconstruction is valuable but prone to microcirculatory instability and ischemia–reperfusion injury (I/R). NIR-IIb (1500–1700 nm) imaging offers deeper penetration and higher SNR than conventional ICG-NIR, potentially enabling earlier detection of perfusion deficits. A manganese-modified single-atom catalyst on NIR-IIb quantum dots (Mn/QD-SAC) combines deep-tissue imaging with broad-spectrum ROS-scavenging enzyme-mimetic activity to mitigate I/R damage.

（2）Hypothesis: Intraoperative and postoperative application of Mn/QD-SAC will enable earlier and deeper detection of perfusion deficits via NIR-IIb imaging and attenuate ROS-driven inflammatory cascades, reducing adipose necrosis and partial flap loss.

（3）Design: Preclinical materials development; in vitro mechanistic assays; small-animal DIEP-like flap I/R model for imaging/therapy; and an observational, minimal-risk human biospecimen sub-study (peripheral blood from DIEP patients) to assess circulating biomarkers.

（4）Primary Endpoints: Flap survival rate at postoperative Day 7; NIR-IIb SNR and correlation with final flap survival area.

（5）Secondary Endpoints: Materials properties; in vitro cell survival/apoptosis/ROS and cytokines; in vivo oxidative stress, inflammation, apoptosis, histology; safety and biodistribution.

（6）Statistics: ANOVA/appropriate nonparametrics for group comparisons; repeated-measures ANOVA; Kaplan–Meier for flap survival; Pearson/Spearman for correlations. Two-sided alpha 0.05.

2. BACKGROUND AND RATIONALE

DIEP flap reconstruction avoids implants and offers natural contour, but intraoperative microcirculatory instability and I/R significantly affect outcomes, with reported double-digit rates of fat necrosis or partial flap loss. I/R triggers a burst of ROS (mitochondrial and enzyme sources such as xanthine oxidase and NOX), causing lipid peroxidation, apoptosis, endothelial dysfunction, and inflammation. Conventional ICG-NIR imaging assists real-time perfusion assessment, yet is constrained by limited depth and autofluorescence. NIR-IIb improves penetration and SNR, better capturing subcutaneous fat and deep microvasculature. Mn/QD-SAC integrates imaging with therapy: single-atom Mn active sites provide ROS-scavenging enzyme-mimetic activity while QDs enable NIR-IIb emission. Pilot evidence in a murine brain injury model supports feasibility for ROS suppression, inflammation inhibition, and tissue repair, warranting systematic evaluation in flap I/R models.

3. OBJECTIVES Primary

（1）Develop and validate in vivo NIR-IIb imaging performance (sensitivity, timing, quantification) of Mn/QD-SAC in DIEP flap I/R and compare to ICG-NIR, laser Doppler/speckle, and tissue oxygenation measurements.

（2）Demonstrate antioxidant, anti-inflammatory, and anti-apoptotic effects and mechanisms (e.g., Nrf2/HO-1, NF-κB, JAK/STAT).

（3）Establish a feasible workflow in murine flap I/R and a clinical preclinical human biospecimen sub-study (DIEP patient peripheral blood) to inform future trials and safety assessment.

4. STUDY DESIGN OVERVIEW

（1）Part A: Materials synthesis and characterization of Mn/QD-SAC (physicochemical, optical, catalytic).

（2）Part B: In vitro mechanistic assays in endothelial, adipose/mesenchymal, and

immune cells under hypoxia–reoxygenation (H/R).

（3） Part C: In vivo murine DIEP-like flap I/R model to test NIR-IIb imaging performance and therapeutic efficacy; safety and biodistribution.

（4） Part D: Human biospecimen sub-study (observational) in DIEP patients for peripheral blood biomarkers at predefined perioperative timepoints; no investigational product administered to humans in this protocol.

5. METHODS

5.1Materials Synthesis and Characterization

（1） Objective: Produce reproducible, stable Mn/QD-SAC and complete physicochemical, optical, and functional characterization.

（2） QD preparation: Ag2Te/Ag2Se-based QDs with controlled size for NIR-IIb emission (1500–1700 nm).

（3）Mn single-atom incorporation: Wet-chemical loading/thermal treatment to achieve isolated Mn sites on QD surfaces; confirm by HAADF-STEM.

（4） Surface modification: mPEG-SH or DSPE-PEG ligand exchange/encapsulation to reduce protein adsorption and enhance circulation; measure zeta potential and solubility.

（5） Characterization: TEM/HAADF-STEM, XPS, XRD, UV–Vis–NIR, and fluorescence spectroscopy (QY, emission peak), ESR for radical scavenging, and stability in serum/salt/protease.

（6） In vitro catalytic activity: SOD-like and CAT-like assays; ESR trapping for •OH and O2•–.

5.2 In Vitro Models and Readouts

（1）Cell types:

Endothelial: HUVEC or HDMEC (barrier function, NO production, apoptosis).

Adipose/mesenchymal: hADSC; adipogenic 3T3-L1 (fat tissue susceptibility to I/R).

Immune: RAW264.7 (M1/M2 polarization, cytokine secretion).

（2）H/R model: 1% O2 hypoxia for 4–8 h followed by reoxygenation (21% O2); monitor ROS dynamics and viability.

（3）Groups: Vehicle; QD without Mn; Mn/QD-SAC low/medium/high doses; positive control NAC (e.g., 5 mM).

（4）Readouts: Cell viability (CCK-8 or CellTiter-Glo), apoptosis (Annexin V/PI), ROS (DCFH-DA, MitoSOX), mitochondrial membrane potential (JC-1), cytokines (ELISA: TNF-α, IL-6, IL-1β), and Western blot (Nrf2, HO-1, p-NF-κB, p-STAT3, iNOS/Arg1).

5.3 In Vivo DIEP-like Flap I/R Model

（1）Model: Murine abdominal perforator-based pedicled or free flap; define ischemia onset; two ischemia durations (2 h and 4 h) for mild/moderate injury.

（2）Randomization (n≈8/group): Sham; I/R+PBS; I/R+QD (no Mn); I/R+Mn/QD-SAC low; I/R+Mn/QD-SAC medium; I/R+NAC.

（3）Dosing (IV tail vein): Imaging 1 mg/kg; therapeutic 5 mg/kg; high-dose 10 mg/kg for toxicity arm.

（4）Timing: Administer at reperfusion; optional repeat at 6 h and 24 h.

（5）Imaging: NIR-IIb intraoperative and at 0 min, 1 h, 6 h, 24 h, 72 h, Day 7, Day 14; record ROI intensity, perfused area, time-to-peak, SNR. Cross-validate with ICG-NIR

(intraop) and laser Doppler/speckle.

（6）Endpoints:

Functional/imaging: Flap survival area (Day 7–14), predictive accuracy of imaging vs final survival.

Molecular/tissue: ROS and oxidative stress (MDA, 8-OHdG, 4-HNE), GSH/GSSG; cytokines (tissue/serum ELISA); IHC (CD31, MPO, MMP-9); TUNEL; Western blot (Nrf2, HO-1, p–NF-κB, p-STAT3).

Safety/biodistribution: CBC; serum chemistries (ALT/AST, creatinine); histopathology (liver/kidney/spleen/lung/heart); ICP-MS of Ag and Mn in blood/organs.

（7）Blinding: Randomization of animals; imaging and histology analyses performed blinded to group.

5.4 Human Biospecimen Sub-study (Observational)

（1）Population: Female, 18–70, undergoing immediate DIEP flap reconstruction after breast cancer surgery; n≈30 convenience sample.

（2）Consent/IRB: Written informed consent before any procedures. Protocol and ICF approved by the hospital ethics committee; amendments submitted for approval.

（3）Data and samples:

De-identified clinical data: age, BMI, operative time, ischemia duration, clinical flap viability, complications.

Peripheral blood draws (10 mL each; serum tube + EDTA) at: pre-op baseline; postoperative 0 h (immediate), 6 h, 24 h, and 72 h.

Analytes:

Hematology/coagulation: CBC, PT, APTT, fibrinogen, D-dimer.

Inflammation/oxidative stress: IL-6, IL-1β, TNF-α, IL-8/IL-10/IL-18, MDA, 8-iso-PGF2α, SOD, CAT, GSH-Px, total antioxidant capacity.

Injury/repair: Lactate, LDH, HMGB1, CK-MB, vWF, VEGF, HIF-1α.

Notes: Results are for research only and not used for clinical decision-making unless an incidental clinically actionable finding is identified and IRB-approved for disclosure.

6. OUTCOME MEASURES

Primary

（1）In vivo therapeutic efficacy: Flap survival rate (%) at Day 7.

（2）In vivo imaging: NIR-IIb SNR and correlation with final flap survival area.

Secondary

（1）Materials: Quantum yield; ROS scavenging efficiency; SOD-like and CAT-like activities.

（2）In vitro: Cell viability; apoptosis; ROS levels; cytokines; pathway proteins.

（3）In vivo biology: MDA, SOD, GSH-Px; cytokines (TNF-α, IL-6, IL-1β, IL-10); histology scores; TUNEL; WB/IHC markers.

（4）Safety: Body weight; CBC; chemistries; organ histopathology; elemental distribution/clearance (ICP-MS).

7. STATISTICAL ANALYSIS PLAN

（1）Analysis populations: All randomized animals analyzed per assigned group. All processed biospecimens are included if predefined QC passes.

（2）Descriptive statistics: Mean±SD or median[IQR] as appropriate; 95% CIs where informative.

（3） Group comparisons: One-way ANOVA with post hoc tests (e.g., LSD-t) for parametric data; Kruskal–Wallis with Dunn's post hoc for nonparametric data.

（4） Repeated measures: Repeated-measures ANOVA with appropriate covariance structure; Greenhouse–Geisser correction if sphericity is violated.

（5） Survival analyses: Kaplan–Meier for flap survival; log-rank test for group differences.

（6） Correlations: Pearson (parametric) or Spearman (nonparametric).

（7） Multiplicity: Control via planned comparisons; exploratory nature acknowledged.

（8） Sample size: Preclinical exploratory study; no formal power calculation. Group size n≈8 informed by prior effect sizes and feasibility. Human sub-study (n≈30) is hypothesis-generating.

（9） Software: SPSS v26.0 and GraphPad Prism v9.0.

（10） Significance: Two-sided alpha 0.05.

8. DATA MANAGEMENT AND QUALITY ASSURANCE

（1） Data capture: De-identified unique study IDs. Source data is maintained securely. Double data entry or programmatic QC where feasible.

（2） Monitoring: Periodic internal review of consent documentation, sample logs, imaging raw files, and analysis scripts.

（3） Archiving: Retain per regulations; controlled destruction or extended retention with IRB approval.

9. PROTECTION OF HUMAN SUBJECTS AND PRIVACY

（1）Consent and ethics: Clear explanation of purpose, procedures, risks/benefits; voluntary participation; IRB approval before initiation; amendments submitted for review.

（2）Privacy: Coded IDs at collection; linkage file stored separately with restricted access. Publications use aggregate or anonymized data only.

（3）Data security: Encrypted institutional servers; role-based access; regular backups; no public cloud for raw PHI. Paper records are stored locked.

（4）Biospecimens: Labeled by ID only; used solely for prespecified assays. Residual samples require new IRB approval and/or renewed consent, or are destroyed per biosafety procedures.

（5）Feedback: Individual lab results are not returned for clinical use unless incidental, clinically actionable findings are confirmed and IRB-approved for disclosure.

10. RISK–BENEFIT ASSESSMENT

（1）Animals: Risks from surgery/anesthesia/agents mitigated by veterinary oversight and humane endpoints; analgesia per IACUC.

（2）Human sub-study: Minimal risk from phlebotomy; no investigational product administered to humans in this protocol. Potential societal benefit from improved understanding of I/R biomarkers and imaging strategies.

11.ETHICAL AND REGULATORY COMPLIANCE

（1）Conduct per Declaration of Helsinki, International Ethical Guidelines, and applicable national regulations and institutional policies.

（2）Animal studies per institutional IACUC standards and relevant guidelines.

12.DISSEMINATION AND DATA SHARING

（1）Results will be disseminated in peer-reviewed publications and conferences.

（2）IPD sharing plan: No sharing of IPD is planned due to patient privacy and ethical considerations, lack of consent for third-party data sharing, institutional/regulatory restrictions, and limited resources for secure de-identification/hosting. Aggregate results will be available in publications or upon request.

13.Administrative Information

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