A. Technical abstract

ParaNano Wound Care, LLC (ParaNano) is developing the Bio-Z™ Smart Bandage, a simple to use wound care product that delivers a visual indication of evolving pathogenic invasion in a burn wound without removing absorbent gauze wound dressings used by DoD during prolonged field care, meeting the objectives of this Topic. The Bio-Z™ Smart Bandage ("the Bio-Z™") enables continuous monitoring of a burn lesion, presents a color-change indicator at critical thresholds of pathogen presence (10^6CFU/cm^2) confirming pathogen invasion, enabling timely action. The Bio-Z™ is a small (2X2cm) bioactive nanofiber membrane intended for placement anywhere on the outer surface of an applied absorbent dressing to monitor a wound for rising pathogen load for up to 72 hours. The Bio-Z™ responds to pathogenic lipase, a biomarker secreted by all ESKAPEE pathogens and some fungal strains. When wound exudate is absorbed into the dressing and pathogen presence exceeds a critical threshold, the Bio-Z™ exhibits a vivid color-change from yellow to green. Detection of all ESKAPEE pathogens, as well as *C. auris* and *C. albicans* was demonstrated under a DHA SBIR Phase I contract awarded in September 2022. The Bio-Z™ is currently at TRL 5 and being advanced toward commercialization. The Bio-Z™ will be registered with the FDA as a Class I bandage in Q2 of 2025 to enable limited market release to support post-market clinical study. The Bio-Z™ will be made available for civilian use initially as a wound care kit that additionally contains a CuraFoam® absorbent dressing and a Tegaderm® transparent adhesive film (shown at right). The pliable, single-use Bio-Z™ Smart Bandage will be a sterilized product individually packaged in foil envelopes (shown center). This Bio-Z™ Smart Bandage Kit will launch through two committed distribution customers targeting use in Long-term Care (LTC) facilities and for patients at home. Initial commercial product launch is focused on underserved, limited-resource areas where lower-skill care providers are the norm. The novel- patented Bio-Z™ is infrastructure independent: no batteries, instrumentation, or specialty training needed, enabling point-of-care use in any environment. Non-invasive and easy-to-use right out of the package, The Bio-Z™ is ideally suited for care of the burn injured Warfighter in limited-resource, prolonged care situations, allowing visual assessment and clinical monitoring without dressing removal; permitting a focus on transporting the higher risk wounded Warfighter to definitive care, while enhancing triage and field care of those who remain in austere environments. The single-use Bio-Z™ is compact and light weight: 20 individually packaged sterile products can fit in a 3"X 2" X 0.25" pouch weighing less than an ounce. Multiple Bio-Z™ placements on the outer layer of an applied absorbent will enable effective monitoring when burn TBSA is extensive. Scaled up manufacturing has yet to be achieved, and Bio-Z™ performance in combination with variable layers of gauze has not been evaluated. To overcome technical risks associated with scaling product manufacturing, and variations in use of absorbents, ParaNano will complete manufacturing validation at scale; sterilization validation, biocompatibility, and shelf-life testing of manufactured products; pre-clinical trials using the FDA Class I product under DoD Clinical Practice Guidelines (CPG); and demonstrate consistent product performance when manufactured at scale.

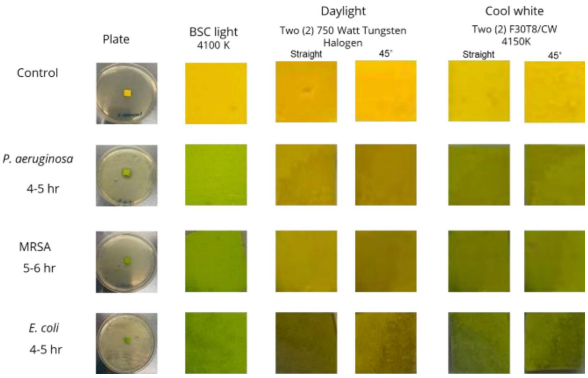
B. Evidence of Phase I feasibility results

ParaNano was awarded a SBIR Phase I contract by DHA [W81XWH22P0133] in September 2022 and completed the contract in May 2023. Key Phase I results: (1) all ESKAPEE pathogen strains were detected within the critical level of presence (i.e., 10^4CFU/cm^2- 10^6CFU/cm^2) triggering a color change, except *E. cloacae* strain BEI 02 that triggered at $1.23x10^7$CFU/cm^2 which exceeded the critical level; and (2) *Candida albicans* and *Candida auris* caused immediate color change due to lipase activity above 10^3CFU/cm^2.

ESKAPEE Pathogens 5 Strains per Bacteria	Color Change 10^4 to 10^6 CFU/cm^2
Enterococcus faecium *Staphylococcus aureus* *Enterobacter cloacae*	2-6 hours
Klebsiella pneumonia *Acinetobacter baumannii* *Pseudomonas aeruginosa* *Escherichia coli*	1-6 hours
Fungal Pathogens	**Color Change** 10^3 to 10^5 CFU/cm^2
Candida albicans *Candida auris*	Under 30mins- 6 hours

The Bio-Z™ Smart Bandage comprises a novel structure of aligned and crossing core-shell nanofibers that cross in a radial pattern and further comprise HCy, a non-toxic lipase-sensitive dye immobilized within the polymeric shell of the core-shell nanofiber. The polymeric shell absorbs moisture from a wound exposing the dye which is cleavable by pathogen secreted lipase when present in the wound. The cleaved dye undergoes a vivid color change response within 6 hours of exposure to bacterial concentrations on the order of 10^6 CFU/cm^2 of wound surface, providing visual notification of rising bacterial load in a wound. Additional prototype development directed toward product commercialization over the time frame following completion of the DHA SBIR Phase I feasibility study produced a number of key results: (1) finalized prototype design, (2) color observability

metrics at various detection times in different light conditions (at right), (3) feasibility of using e-beam terminal sterilization on the Bio-Z™ Smart Bandage, (4) production machine optimization, and (5) Standard Operating Procedures and design controls required for FDA Class I registration. Given the simplicity of a color change indication, evolving pathogenic invasion can be readily assessed by care providers in any environment. No electronics or culturing is needed, enabling use in an austere environment. By allowing the dressing to stay in place absent signs of colonization, resources and personnel time saved can be used to care of other injured persons.



C. Phase II technical objectives and key results

Objective I. A Bio-Z™ Smart Bandage product refined for use with current DoD Clinical Practice Guidelines
Key Deliverable/Result: verification of product safety and efficacy when using standard DoD dressings
Objective II. Pre-clinical trial using an in vivo model of an infected/inflamed partial- or full thickness burn wound
Key Deliverable/Result: Study report demonstrating clinically significant results in a live setting
Objective III. Validation of Bio-Z™ membrane production at scale using Good Manufacturing Practice (GMP)
Key Deliverable/Result: Validated GMP manufacturing process per FDA requirements
Objective IV. Efficacy verification of e-beam sterilization for case-lot, packaged product at scale
Key Deliverable/Result: Validation of Bio-Z™ Smart Bandage sterility and effectiveness after terminal sterilization of palletized, case-lot product quantities; deliver advanced prototypes to DHA
Objective V. Demonstrated consistent performance of the Bio-Z™ Smart Bandage
Key Deliverable/Result: Verification of stability, shelf-life, sensitivity, observability, and response time

Task 1. Hold kick-off meeting with the DHA TPOC to gain consensus on the timeline and key results to achieve
Task 2. Conduct in vitro testing of the FDA Class I registered Bio-Z™ Smart Bandage applied over "clean dry gauze" following current DoD Clinical Practice Guidelines (CPG) to establish a baseline response to relevant pathogens
Task 3. Evaluate efficacy of the FDA Class I registered Bio-Z™ Smart Bandage in a pre-clinical trial testing pathogens of greatest concern using a relevant in vivo model of an infected/inflamed partial- or full thickness burn wound
Task 4. Complete manufacturing validation for scaled capacity production (i.e., 1.4 million membranes)
Task 5. Conduct biocompatibility testing per FDA requirements, sterilization process testing of products manufactured at scale, and product shelf-life evaluation (i.e., membrane & packaging).
Task 6 Refine the product (i.e., Bio-Z™ Smart Bandage and packaging) as may be needed for DoD use
Task 7. Demonstrate consistent performance of the Bio-Z™ Smart Bandage using the ParaNano bench testing and quality control procedures, and deliver no less than 4 prototypes (packaged) to DHA for military evaluation
Task 8. Engage with FDA and develop a transition to Phase III plan including regulatory and funding strategy.

D. Commercialization strategy

The Bio-Z™ Smart Bandage Kit is being commercialized for civilian use as an FDA Class I product with an initial focus on chronic wounds afflicting limited-resource communities underserved in the U.S. healthcare system. ParaNano has two committed customers for deployment of this product into 1,300+ LTC facilities across the U.S. and into the VA system nationally. ParaNano is now establishing pilot manufacturing capability at the Meridian Technology Center Incubator in Stillwater, OK. This will enable manufacturing validation and produce finished products that will support testing required for FDA registration and this Phase II project. ParaNano has received quotes and selected the laboratory service providers needed to conduct biocompatibility and sterilization process testing, and has engaged a regulatory consulting firm to provide guidance and manage the validation processes necessary for self-registration in 2025 with the FDA as a Class I bandage. The Bio-Z™ is uniquely produced using a patented process executed on proprietary electrospinning machines comprising a modular system that can be incrementally expanded to increase the scale of product manufacturing. Ten (10) machines are planned for the pilot facility which will enable production of 1.4 million Bio-Z™ Smart Bandage Kits per year. ParaNano has engaged with WIC Products, LLC to expand production capacity utilizing a 5400 ft^2 clean room space located in Oklahoma City which will support scaling production capability to over 14 million Bio-Z™ Smart Bandage Kits in 2026. ParaNano is currently seeking both dilutive and non-dilutive funding to advance commercialization of the Bio-Z™ Smart Bandage.