**ABSTRACT**

Background: Burn injuries sustained from explosions and fires account for 5-10% of combat casualties and multidrug-resistant infections are growing at an alarming rate. Silver-containing products such as silver sulfadiazine (SSD) have long been considered the standard topical therapy for partial burns, however, the efficacy of these treatments for improving mortality, wound healing, and decreasing infection rates has been questioned and novel treatments are urgently needed. Exogenous nitric oxide has been investigated as an antimicrobial agent due to its broad-spectrum activity and ability to attack multiple biologically essential targets, making the development of resistance unlikely. Novan’s proprietary technology stably stores the gaseous species as an engineered macromolecule allowing control of nitric oxide release.

**RESULTS**

**NVN4428**

**PhoGel48**

**1:1**

NVN4428 Ointment : Hydrogel

Figure 2. Experimental Design of Deep Partial Thickness Wound Study.

Table 1. Bacterial Efficacy Against Planktonic and Biofilm-Embedded Multidrug-Resistant Microorganisms.

**N.spinifex** demonstrated a dose responsive reduction in bacterial load that was significantly more efficacious compared to Silver Sulfadiazine (B). In vivo efficacy of topical formulations was assessed in a porcine partial thickness infected wound model (C). A statistically different from vehicle at corresponding timepoint. *statistically different from silver at designated timepoint.

**MATERIALS & METHODS**

**Time Kill Assays:** Time Kill assays were performed in accordance with the ASTM E2915 Standard. Varying concentrations of antimicrobial agents (silver sulfadiazine or NVN4428) were incubated with each microorganism (10° CFU/ml) in triplicate (100 μl, pH 7.5±0.5, 37°C). Uninoculated cultures were included as controls. At 1 and 4 hours of incubation samples were taken and serial dilutions were plated to determine the number of viable bacteria.

**Minimum Biofilm Eradication Concentrations:** The minimum biofilm eradication concentration (MBEC) for A. baumannii, C. albicans, and Staphylococcus aureus were determined using the MBEC assay plate (Innovotech) routinely used for high throughput screening of biofilm-embedded bacteria. Biofilms were grown for 24 hrs at 37°C prior to challenge with NVN4428 and silver sulfadiazine. Biofilms were challenged with antimicrobial agents for 18 hours and then sonicated for 25 minutes in recovery media to disperse the biofilm bacteria for colony counting. Recovered biofilms were grown for 18 hrs at 37°C and bacterial viability was subsequently determined by MTT assay. Concentrations completely inhibiting growth of recovered bacteria are reported.

**Deep Partial Thickness Wound Study:** Three specific pathogens (Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans) were inoculated into burn wounds (10° CFU/ml) that were subsequently treated with silver sulfadiazine or NVN4428. Wounds were treated with 15 mg/cm² of each test article at 0, 24, and 48 hours post-treatment. Bacterial load was assessed at 24 hours post-treatment. Biopsies were taken from the wounds after 48 hours of treatment and a bacterial load was assessed.

**Biofilm Assays:** The ability of surface-attached cells to grow and form a biofilm was determined using the MBEC assay plate. Biofilms were grown for 24 hrs at 37°C for each test article. The MBEC assay plate was incubated at 37°C for 24 hours with each test article. The plate was then incubated at 37°C for 24 hours with each test article. The plate was then washed with 100 μl of sterile water and plated on blood agar plates. The number of colonies was determined and the concentration of each test article that inhibited biofilm formation was recorded.

**NC:** Not considered significant.

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**Novan Therapeutics │ 4222 Emperor Boulevard, Suite 200, Durham, NC 27703 │ 919-485-4080 │ www.novantherapeutics.com**