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Nanoemulsion Therapy for Burn Wounds is Effective as a Topical Antimicrobial Against Gram Negative and Gram Positive Bacteria

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Abstract

Objective—The aim of this study is to investigate the antimicrobial efficacy of two different nanoemulsion formulations against Gram positive and Gram negative bacteria in an *in vivo* rodent scald burn model.

Methods—Male Sprague-Dawley rats were anesthetized and received a partial-thickness scald burn. Eight hours following burn injury the wound was inoculated with 1×10^8 colony forming units of *Pseudomonas aeruginosa* or *Staphylococcus aureus*. Treatment groups consisted of two different nanoemulsion formulations (NB-201, NB-402), nanoemulsion vehicle (NE vehicle), or saline. Topical application of the treatment was performed at 16 and 24 hours after burn injury. Animals were euthanized 32 hours after burn injury and skin samples obtained for quantitative wound culture and determination of dermal inflammation markers. In a separate set of experiments, burn wound progression was measured histologically after 72 hours of treatment.

Results—Both nanoemulsion formulations (NB-201, NB 402) significantly reduced burn wound infections with either *Pseudomonas aeruginosa* or *Staphylococcus aureus*, and decreased median bacterial counts at least 3 logs as compared to animals with saline applications ($p < 0.0001$). NB-201 and NB-402 also decreased dermal neutrophil recruitment and sequestration into the wound as measured by myeloperoxidase assay and histopathology ($p < 0.05$). In addition, there was

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Conflict of Interest Disclosure:

Vladislav A. Dogachev: None

Susan M. Ciotti: Employee of NanoBio Corporation

Rone Eisma: Employee of NanoBio Corporation

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a reduction in the pro-inflammatory dermal cytokines (IL-1 β , IL-6 and TNF- α) and the neutrophil chemoattractants CXCL1 and CXCL2. By histology examination, both NB-201 and NB-402 appeared to suppress burn wound progression 72 hours after injury.

Conclusions—Topically applied NB-201 and NB-402 are effective in decreasing Gram positive and negative bacteria growth in burn wounds, reducing inflammation and abrogating burn wound progression.

Keywords

Burn wound; burn inflammation; burn wound infection; nanoemulsion; nanotechnology; topical therapy; wound healing

Introduction

Thermal burns are a common and devastating form of traumatic injury. Approximately 100,000 individuals are hospitalized with moderate to severe burn injuries each year and 5,000 of these patients die from burn related complications [1]. Burn wound progression is a process by which an initial partial-thickness burn injury advances to a deep partial-thickness or full-thickness burn wound over time. The cause of burn wound progression is multifactorial, but involves the host response and dermal inflammation [2, 3]. Progression of a burn wound is an important phenomenon because wound depth is the principal determinant of morbidity and need for surgical intervention, which involves operative excisional debridement of the dead tissue and split thickness skin grafting to restore the patient's skin integrity [4]. This is a painful, labor-intensive treatment regimen that results in long periods of hospitalization during which the patient is vulnerable to infection. It can also disfigure the patient and cause long term problems with scarring and burn wound contracture [5].

In contrast, a partial-thickness burn wound that does not progress is capable of self-healing with minimal scarring. In this situation, the skin regenerates from the undamaged epithelial cells that line the shaft of each hair follicle through coalescence of epithelial skin islands. Development of a topical antimicrobial therapy that ameliorates burn wound progression without causing cellular damage and harmful inflammation would represent a significant breakthrough in the treatment of thermal burn injury. This treatment, if successful, could lessen the need for skin grafting, speed recovery time, result in fewer infectious complications, and improve functional outcomes for many burn patients.

Nanoemulsion formulations are proven to exhibit broad antimicrobial properties and are ideally suited for the treatment of burn wounds. These oil-in-water emulsions are made using high shear conditions to produce droplets that physically fuse with the lipids in microbial outer membranes, leading to membrane destabilization and lysis of pathogens [6–9]. NB-201 consists of water, refined soybean oil, an organic solvent, nonionic surfactant, and a cationic surfactant. The cationic surfactant is located at the oil-water interface and serves to provide a positive charge to the nanoemulsion droplet. The principle of like charges repelling stabilizes the nanoemulsion droplets in solution and prevents them from coalescing.

In a rodent model, we previously used a topically applied, antimicrobial nanoemulsion formulation to treat burn injured skin [9]. This nanoemulsion formulation (NB-201), resulted in 3 log decreases in Gram negative bacterial counts in the burn wound and significantly reduced dermal levels of pro-inflammatory cytokines. By lowering excess influx of neutrophils into the burn wound and modulating the pro-inflammatory state, NB-201 appeared to attenuate burn wound inflammation in the early post-injury phase.

In the present experiments we sought to test *in vivo* two different nanoemulsion formulations that have been optimized *in vitro* for antimicrobial activity and validate our prior findings. NB-201, the previously tested compound, consists of benzalkonium chloride as the cationic surfactant and Tween 20 as the nonionic surfactant. NB-402 is similar to NB-201 but contains cetylpyridinium chloride instead of benzalkonium chloride and Poloxamer 407 (P407) in place of Tween 20. Our objective was to test these formulations for *in vivo* antimicrobial efficacy against both Gram negative and positive bacteria and evaluate dermal inflammation and wound healing following partial-thickness burn in the rat scald burn model.

Materials and Methods

Reagents

Unless otherwise indicated, all reagents were purchased from Sigma-Aldrich Corp. (St. Louis, MO).

Animals

Male specific pathogen-free Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 250 to 300 g were used. All experiments were performed in accordance with the National Institutes of Health (NIH) guidelines for care and use of animals. Approval for the experimental protocol was obtained from the University of Michigan Animal Care and Use Committee.

Nanoemulsion Formulation

The nanoemulsions (NB-201 and NB-402) were prepared by emulsification of a cationic surfactant, a nonionic surfactant, ethanol, a chelating agent, refined soybean oil, and water using proprietary methodology in accordance with good manufacturing practices (GMP). Two different cationic surfactants were used in this study. Benzalkonium chloride (BAC) was the cationic surfactant incorporated in NB-201 formulation with Tween 20 as the nonionic surfactant. Cetylpyridinium chloride (CPC) was incorporated in the NB-402 formulation with P407 as the nonionic surfactant. The nanoemulsion vehicle (NE vehicle) was prepared in a similar fashion, without incorporation of any cationic surfactant and P407 as the nonionic surfactant. The surfactants, both cationic and nonionic, reside at the interface between the oil and water phases. The hydrophobic tail of the surfactant distributes in the oil core and its polar head group resides in the water phase as shown in Figure 1.

Mean Particle Size Analysis and Polydispersity Index

Mean particle size (Z-average) and polydispersity index (PdI) were determined for each nanoemulsion formulation. The particle size and PdI of the sample was measured by photon correlation spectroscopy using a Malvern Zetasizer Nano ZS90 (Malvern Instruments, Worcestershire, UK). The nanoemulsion formulations mean particle sizes and polydispersity index are shown in Table 1.

Burn Model

The scald burn procedure was performed according to a previously established method to produce a partial-thickness burn injury [9]. In brief, animals were anesthetized with a intraperitoneal injection of sodium pentobarbital (Nembutal; Abbott Laboratories, North Chicago, IL). Dorsal hair was closely clipped and then removed using depilatory cream (Nair; Church & Dwight Inc., Princeton, NJ). Each rat was placed in a mold, which exposed the dorsal region over 20% of the total body surface area. Partial-thickness scald burn injury was achieved by immersing the exposed skin of the rat in a 60°C water bath for 25 seconds. The burn wound was debrided with dry sterile gauze and rinsed with saline. Each animal was resuscitated with 10 mL Ringer's lactate solution (5 mL intraperitoneal and 5 mL subcutaneously). After drying, an occlusive dressing of sterile Telfa (Kendall Co., Mansfield, MA) and Tegaderm HP (3M HealthCare, St Paul, MN) was applied to prevent wound contamination. Following burn injury each rat was individually housed and received subcutaneous injections of 0.01 mg/kg buprenorphine solution at time 0, 8, 16 and 24 hours post-burn for pain control.

Bacterial Culture and Wound Inoculation

Pseudomonas aeruginosa (*P. aeruginosa*) isolated from a burn patient was provided by the Department of Pathology at the University of Michigan and used in this study. This bacterial isolate is sensitive to the topical agent Silvadene and Sulfamylon. A bacterial inoculum was prepared by thawing a frozen aliquot in 40 mL of Trypticase soy broth (Becton Dickinson, Franklin Lakes, NJ) and grown overnight at 37°C with constant shaking. A sample of the stationary phase culture was transferred into fresh Trypticase soy broth and incubated for 2.5 hours to reach the log phase. This subculture was centrifuged, washed with saline, and re-suspended in 10 mL of ice-cold saline. The optical density of the suspension was measured at 620 nm, and bacterial concentration (CFU/mL) was calculated using the formula $OD_{620} \times (2.5 \times 10^8)$. The bacteria were diluted with saline to a final concentration of 1×10^8 CFU per 200 μ L. At 8 hours after burn injury, animals were anesthetized with inhaled isoflurane and underwent topical application of 1×10^8 CFUs of log-phase *P. aeruginosa* pipetted onto a piece of Telfa in a uniform fashion. The bacteria inoculated Telfa was applied to the previously burned skin and covered with a Tegaderm occlusive dressing.

Staphylococcus aureus (*S. aureus*) was obtained from ATCC (catalog number 4012). A bacterial inoculum was prepared by thawing a 100 μ L aliquot (stored in 0.5 mL, stored in 50% skim milk at -80°C) and inoculated into Trypticase soy broth overnight at 37°C. Washing, resuspension, and dilution was performed in the same manner as described above to a concentration of 1×10^8 CFU per 200 μ L. At 8 hours after burn injury rats were

anesthetized and underwent inoculation with *S. aureus* similar to the method described for *P. aeruginosa*

Topical Burn Wound Treatment

Nanoemulsion formulations, (NB-201, NB-402) and the NE vehicle were obtained from NanoBio Corporation (Ann Arbor, MI). Each experimental group consisted of animals with a burn injury inoculated with bacteria and treated topically with one of the following: a) sterile saline solution, b) NE vehicle, c) NB-201, or d) NB-402. At 16 hours after burn injury, animals were anesthetized with inhaled isoflurane. The occlusive dressing and Telfa were removed and 2 mL of sterile saline solution, NE vehicle, NB-201, or NB-402 was applied in a uniform fashion to the burn wound surface using a spray bottle. The burn wound was covered with a Telfa and Tegaderm occlusive dressing. This treatment and dressing change was repeated at 24 hours after burn injury. The antimicrobial activity experiments were terminated 32 hours after burn injury.

Saline and NE vehicle were both used as control treatments. Saline was selected since it is an isotonic liquid and has no antimicrobial properties. The nanoemulsion formulations consist of multiple reagents mixed together in a proprietary manner to create a functional high energy emulsion with antimicrobial activity. The NE vehicle was created using the same manufacturing method but omitting the cationic surfactant. In these experiments the NE vehicle contained P407 as the nonionic surfactant.

In a separate set of experiments, burn wound progression was examined. The experimental groups consisted of animals with a burn injury only and no inoculation with bacteria. Burned rats were treated topically with one of the following starting 8 hours after scald injury: a) sterile saline solution, b) NE vehicle, c) NB-201, and d) NB-402. The burns were covered with Telfa and a Tegaderm occlusive dressing. Dressings were changed and burn wound treatment occurred at 16, 24, 48, and 60 hours. Digital pictures were taken at the time of each dressing change to monitor healing progression. The experiment was terminated at 72 hours.

Tissue Harvest

At the selected endpoint after thermal injury the animals were euthanized and skin tissue samples were harvested using sterile technique. Skin samples were used immediately or frozen in liquid nitrogen.

Quantitation of Bacterial Wound Infection

A 100 mg piece of excised full thickness skin tissue was mechanically homogenized in 1 mL of sterile saline solution. This homogenate was then further diluted with 9 mL of sterile saline solution. Serial dilutions were performed, and skin homogenates were plated in triplicate on blood agar plates (Becton Dickinson). Culture plates were incubated for 24 hours at 37°C and CFUs were counted [10].

Quantitation of Soluble Mediators by ELISA

A 100 mg piece of excised full thickness skin tissue was mechanically homogenized in 1 mL of sterile Phosphate Buffered Saline (1X), pH 7.4, containing 0.01% (w/v) Triton X (Roche, Indianapolis, IN) and complete protease inhibitors cocktail (Complete X). This homogenate was centrifuged at 3000 g for 5 minutes at 4°C and used for ELISA. Rat interleukin 1-beta (IL-1 β), interleukin 6 (IL-6), tumor necrosis factor alpha (TNF- α), CXCL1 cytokine-induced neutrophil chemoattractant-1 (CINC-1, rat GRO, or rat KC) and CXCL2 or cytokine induced neutrophil chemoattractant-3 (rat MIP2, GRO β) were measured by sandwich enzyme-linked immunosorbent assay (ELISA) using DuoSets from R&D Systems Inc. (Minneapolis, MN). Myeloperoxidase levels were determined using an ELISA kit from Hycult biotech (Plymouth Meeting, PA). For the rat myeloperoxidase (MPO) assay, skin tissue was homogenized in 1 mL of sterile Phosphate Buffered Saline (1X), pH 7.4, containing complete protease inhibitors cocktail (Roche).

Histology

Fresh 4mm full thickness skin tissue biopsies were fixed in 10% buffered formalin and embedded in paraffin. Sections 4 μ m thick were sliced and affixed to slides, deparaffinized, and stained with hematoxylin and eosin to assess morphological changes. To evaluate neutrophil infiltration into the burn wound we counted neutrophils within a 1mm x1mm microscope grid at high power (40x magnification) for six fields per slide of full thickness skin and an average value of cells/mm³ was determined for each burn wound sample. Skin histology samples were scored for burn injury by an independent and blinded pathologist using the following system: distribution of cellular infiltrate (0=none, 1=focal, 2=multifocal, 3=locally extensive, 4=multifocal and locally extensive, 5=diffuse), inflammation severity (0=none, 1=mild, 2= moderate, 3=severe), infiltrate type (0=none, 1=acute, 2= subacute, 3=chronic), necrosis (0=none, 1=minimal, 2=moderate, 3= severe). A final score was computed by summing the scores in each subdivision.

Statistical Methods

All statistical analysis and graph(s) were performed using GraphPad Prism software, version 5.0 (GraphPad Software, La Jolla, CA). Results are presented as mean values $\pm$ the standard deviation (SD) unless otherwise noted. Continuous variables were analyzed using a one-way analysis of variance (ANOVA) and Tukeys post-test comparisons. The Kruskal-Wallis test with Dunn's multiple comparisons was used to evaluate differences in medians for data with a non-parametric distribution. Statistical significance was defined as a *p*-value < 0.05.

Results

Topical treatment of *Pseudomonas aeruginosa* inoculated thermal burn injury with either NB-201 or NB-402 reduces wound bacterial counts, dermal cytokine levels and neutrophil sequestration

After partial thickness burn injury and inoculation of the wound with *P. aeruginosa* 33 μ g/cm² of NB-201 or NB-402 was applied. Application of NB-201 decreased the CFU's of *P. aeruginosa* (colony forming units cultured per gram of skin tissue) by 4 logs compared to

the saline treated group (Figure 2). A similar bacterial count reduction was found using the NB-402 formulation. These counts were below the clinical threshold considered to be indicative of burn wound infection of 1×10^5 CFU's/g tissue [11], and indicated a significant antimicrobial effect from both emulsions.

Skin homogenates from the NB-201 and NB-402 treated wounds showed non-significant changes in dermal levels of IL-1 β , IL-6, and TNF- α compared to saline or NE vehicle treated controls 32 hours after burn injury in the presence of *P. aeruginosa* infection (Figure 3). In contrast, NB-201 did markedly suppress the concentrations of the neutrophil chemoattractants CXCL1 and CXCL2, as well as myeloperoxidase levels compared to saline treated wounds. NB-402 significantly reduced the mean level of CXCL2, but not CXCL1 and again reduced the myeloperoxidase levels compared to the saline treatment.

Topical treatment of *Staphylococcus aureus* inoculated thermal burn injury with either NB-201 or NB-402 reduces wound bacterial counts, dermal cytokine levels and neutrophil sequestration

NB-201 and NB-402 also exhibited antimicrobial efficacy against *S. aureus* inoculated into partial thickness burn wounds 8 hours after injury. NB-201 and NB-402 treatment in a manner identical to that used with the *Pseudomonas* wounds significantly reduced median quantitative *S. aureus* cultured bacterial counts 4 logs as compared to saline treated burns (Figure 4). The dermal cytokine profile was, however, slightly different following nanoemulsion treatment of *S. aureus* inoculated burn wounds as compared to the *P. aeruginosa* infected animals. In the *S. aureus* inoculated wounds, there was a significant reduction in IL-1 β , IL-6, TNF- α , and CXCL1 for the NB-402 treated animals compared to the saline group, but not for IL-1 β or CXCL1 in NB-201 treated wounds (Figure 5). Although the CXCL1 chemokine level was decreased with the control nanoemulsion (NE vehicle), only treatment with NB-201 or NB-401 resulted in diminished dermal neutrophil recruitment as documented by decreases in myeloperoxidase.

Either NB-201 or NB-402 limit dermal inflammatory cytokine production and neutrophil sequestration and attenuates burn wound progression in sterile, partial-thickness burns

In rats with partial-thickness burns but no bacterial infection, burn wounds were treated with the experimental nanoemulsions or controls at 8, 16, 24, 48 and 60 hours after injury. Digital photographs were taken at 48 and 72 hours after the burn injury. Rats receiving cationic surfactant containing nanoemulsions (NB-201 or NB-402) experienced less discomfort compared to saline or NE vehicle groups as none of these animals attempted to disrupt their wound dressing. The saline and NE vehicle treated animals demonstrated significant burn wound progression and skin ulcerations compared to either NB-201 or NB-402 treated wounds; this appeared to be influenced by all of the rats in the control groups removing or attempting to remove the dressings despite ongoing analgesic administration (Figure 6). Although we originally planned to follow wound healing for up to 14 days in these animals, the study was truncated at 72 hours due to the difficulty in keeping the wound dressings intact in the saline and NE vehicle treated rats.

Levels of dermal cytokines measured in skin homogenates were lower in the non-infected burn wounds as compared to those in burn wounds inoculated with bacteria (Figure 7A). Despite this, we observed significantly decreased levels of IL-1 β and TNF- α in burned skin for both NB-201 and NB-402 treated animals compared to the saline and NE vehicle groups. Chemokine levels (CXCL1 and CXCL2) were reduced for NB-201 and NB-402 treated animals compared to the saline group. Once again, NB-201 and NB-402 treatment significantly diminished myeloperoxidase levels as compared to controls. Histopathologic counting of neutrophils present in skin samples demonstrated a reduced infiltration of neutrophils into the burned skin treated with NB-201 and NB-402 vs. the saline or NE vehicle treated animals (Figure 7B). These findings suggest that treatment with NB-201 or NB-402 resulted in lessened dermal neutrophil recruitment and sequestration into the burn wound compared to the saline and NE vehicle controls.

Evaluation of skin samples with a histopathology scoring system revealed significantly less burn injury at 72 hours after treatment with NB-201 or NB-402 compared to saline treated controls (Figure 8). The NB-201 or NB-402 treated rats also maintained their pre-burn injury measured body weight, whereas the saline or NE vehicle treated animals lost body mass over the 72 hours of treatment. Application of NB-201 or NB-402 reduced burn wound progression and these animals did not demonstrate histologic changes consistent with increasing burn wound depth.

Discussion

In these experiments we found that both NB-201 and NB-402 are effective at decreasing the numbers of Gram negative and positive bacteria in contaminated, partial-thickness burn wounds. NB-201 reduced the levels of the chemotaxis mediators CXCL1 and CXCL2 in burn wounds with Gram negative bacteria and IL-6 and TNF α in burn wounds with Gram positive bacteria. NB-402 decreased levels CXCL2 in burn wounds with Gram negative bacteria and IL-1 β , IL-6, TNF α , and CXCL1 in burn wounds with Gram positive bacteria. NB-201 and NB-402 were also extremely effective in abolishing neutrophil recruitment into burn wounds as assessed by MPO assay and histology. Cross sectional histology scoring of non-infected partial-thickness burn wounds demonstrated a reduction in burn injury to the skin treated with NB-201 or NB-402 as compared to saline or NE vehicle treated controls and therefore may have the potential to reduce the progression of a partial-thickness burn injury. A serendipitous finding was that topical nanoemulsion therapy appeared to support a more normal rat homeostasis in the presence of burn injury as evidenced by the maintaining of body mass. This study confirms our previous results with NB-201 [9] and showed that either the NB-201 or NB-402 formulation can be utilized as broad spectrum topical antimicrobial agents following burn injury

We tested two different cationic surfactants as active antimicrobial agents in our nanoemulsion formulations; benzalkonium chloride and cetylpyridinium chloride. Benzalkonium chloride is a common preservative in pharmaceuticals and personal care products such as eye, ear, and nasal drops. The greatest biocidal activity is associated with the C12 dodecyl and C14 myristyl alkyl derivatives. The mechanism of bactericidal/microbicidal action is believed to be from dissociation of the cellular membrane lipid bilayer

which induces leakage of cellular contents [12]. Cetylpyridinium chloride is a cationic quaternary ammonium compound used in mouthwashes, toothpastes, lozenges, throat, breath, and nasal sprays. It is an antiseptic that kills bacteria and other microorganisms [13]. The nanoemulsion droplet size was similar for both formulations and while the CPC molecule has a longer hydrophobic tail than BAC; however BAC has a larger polar cationic head group. NB-402 has P407 as the nonionic surfactant, while NB-201 uses Tween 20, and P407 is bulkier in structure than the Tween 20 structure. Removing the cationic surfactant from the composition substantially affects the antimicrobial activity as evidenced by marginal bactericidal activity of the NE vehicle. Thus, while the surfactants were necessary for antimicrobial activity, there did not appear to be significant differences between the activities of these formulations based on different surfactants.

Topical antimicrobial therapy is a mainstay of burn wound treatment. Current available topical agents consist of silver sulfadiazine, mafenide acetate, and nanocrystalline impregnated dressings. None of these agents is known to act to reduce burn wound inflammation and inhibit burn wound progression. In our prior work with NB-201 and partial-thickness burns we demonstrated that this nanoemulsion could reduce the degree of hair follicle epithelial cell apoptosis in the early post-burn period [9]. In these experiments both NB-201 and NB-402 treated animals had visual evidence of decreased burn wound progression. Control of dermal inflammation and the resultant systemic inflammatory response is a promising approach to reduce burn wound progression. Topical application of SB 202190, an inhibitor of p38 mitogen activated protein kinase, has been shown to reduce the level of inflammation at the dermis [14]. This diminished inflammation was associated with reduced neutrophil sequestration, microvascular damage, and epithelial apoptosis in burn wound hair follicle cells [14]. NB-201 and NB-402 appear to act in a manner similar to SB 202190 by diminishing inflammation and apoptosis, but offer the added benefit of being substantially antimicrobial. The mechanism of the anti-inflammatory effect of the nanoemulsion appears separate from its antimicrobial activity, since it also occurred in sterile wounds, and deserves further evaluation. Oil rich nanoemulsion formulations also appear to reduce skin irritation for a given level of cationic surfactant that otherwise would be irritating to the skin.

Nanotechnology uses the unique properties of engineered objects that function as a unit within the overall size range of 1–1,000 nanometres. Nanostructure materials, including nanoparticles, nanoemulsions or nanotubules can be used to effectively manipulate or deliver immunologically active components to target sites in the setting of infectious or non-infectious diseases [15]. Nanoemulsion compounds are inexpensive to manufacture, have an excellent safety profile, and have been applied topically in over 2,000 subjects with herpes labialis or onychomycosis. In patients with daily usage for up to 9 months there was no significant skin irritation, no withdrawals due to treatment related adverse events, and no serious treatment related adverse events [16]. The nanoemulsion formulation used in these clinical trials is similar to the NB-201 and NB-402 formulations studied in these experiments.

The selection of an animal model for investigation of burn injury depends on a number of factors including availability, cost, ease of handling, investigator familiarity, and

anatomical/functional similarity to humans. Small mammals are frequently used for wound healing studies; however, these mammals differ from humans in that their wounds often heal by secondary contraction and not re-epithelization [17–19]. A porcine model of burn injury is available and is an excellent tool for the evaluation of therapeutic agents destined for use in human wounds and burns [20–24]. In future experiments, we plan to examine the effectiveness of topical antimicrobial nanoemulsion formulations on abating burn wound progression, promoting wound healing, and assessing for acute systemic toxicity.

Conclusion

Topical nanoemulsion therapy significantly reduces Gram positive and negative bacterial growth in burn wounds. In addition, NB-201 and NB-402 are capable of reducing dermal inflammation and burn wound progression. Nanoemulsion therapy represents a promising treatment for early burn wounds. Development of a topical antimicrobial treatment that also modulates the skin's inflammatory response to burn injury and prevents conversion of a partial-thickness burn to full-thickness injury could significantly improve early treatment for burn patients.

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Interphase: Surface between the water and oil

Cationic Surfactant (e.g. Cetylpyridinium chloride: CPC)
Nonionic Surfactant

Core: oil

Oil (e.g. Soybean oil)

Figure 1.
Schematic illustration of an oil-in-water nanoemulsion droplet.

Figure 2.

Topical application of NB-201 and NB-402 inhibits *Pseudomonas aeruginosa* growth in burn wounds. The scatter plot represents cultured CFUs for each individual animal. The median value for each group is plotted as a horizontal line. N=9 animals per group. $p=0.0001$, Kruskal-Wallis test, * $p < 0.05$ vs. Saline, Dunn's multiple comparison test. # $p < 0.05$ vs. NE vehicle, Dunn's multiple comparison test.

Figure 3.

Topical application of NB-201 and NB-402, after partial thickness burn injury and *Pseudomonas aeruginosa* infection, decreases production of CXCL1, CXCL2 and myeloperoxidase. * $p < 0.05$ vs. Saline, one-way ANOVA with Tukey's multiple comparison test. # $p < 0.05$ vs. NE vehicle, one-way ANOVA with Tukey's multiple comparison test.

Figure 4.

Topical application of NB-201 and NB-402 inhibits *Staphylococcus aureus* growth in burn wounds. The scatter plot represents cultured CFUs for each individual animal. The median value for each group is plotted as a horizontal line. N=6 animals per group. $p=0.0002$, Kruskal-Wallis test, * $p < 0.05$ vs. Saline, Dunn's multiple comparison test.

Figure 5.

Topical application of NB-201 and NB-402 after partial thickness burn injury and *Staphylococcus aureus* infection decreases production of dermal pro-inflammatory cytokines and neutrophil recruitment/sequestration. N=6 animals per group. * $p < 0.05$ vs. Saline, one-way ANOVA with Tukey's multiple comparison test. # $p < 0.05$ vs. NE vehicle, one-way ANOVA with Tukey's multiple comparison test.

Figure 6.

Photographs (A–H) and cross-sectional histology (I–L) analysis of burned skin after treatment with Saline, NE vehicle, NB-201 or NB-402. Digital photos (A–H) of burned skin from Saline (A and E) and NE vehicle (B and F) treated rats demonstrate progression of the partial thickness burn and evidence of ulceration. The nanoemulsion treated skin showed minimal evidence of burn wound progression and appeared similar to partial thickness burned skin at time 0 hours (NB-201 (C and G) and NB-402 (D and H)). Histological analysis (I–L), hematoxylin and eosin stain, original magnification 60x, revealed loss of epidermis in Saline (I) and NE vehicle (J) treated groups. The epidermis and dermis remained intact in NB-201 (K) and NB-402 (L) treated groups. Representative sections from two independent studies are shown. N=6 animals per group.

Figure 7.

A: Topical application of NB-201 and NB-402 after partial thickness burn injury in the absence of infection decreases production of dermal pro-inflammatory cytokines and myeloperoxidase. B: Histopathology detailing neutrophil counts per slide. N=6 animals per group. * $p < 0.05$ vs. Saline, one-way ANOVA with Tukey's multiple comparison test. # $p < 0.05$ vs. NE vehicle, one-way ANOVA with Tukey's multiple comparison test.

Figure 8.

Histology scores and mean body mass. N=6 animals per group. * $p < 0.05$ vs. Saline, one-way ANOVA with Tukey's multiple comparison test. # $p < 0.05$ vs. NE vehicle, one-way ANOVA with Tukey's multiple comparison test.

Table 1

Composition, mean particle size and polydispersity index of the oil-in-water nanoemulsions.

Formulations	Type of Cationic/Nonionic Surfactant	Mean Particle Size (nm)	Polydispersity (Pdl) Index
NB-201	BAC/Tween 20	257.0 ± 1.7	0.081 ± 0.031
NB-402	CPC/P407	212.1 ± 2.7	0.103 ± 0.025
NE Vehicle	None/P407	336.2 ± 12.8	0.135 ± 0.034