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In vitro bactericidal efficacy of a new sun- and heat burn gel

Lucie Martineau [a,*], Hans-Michael Dosch [b,c]

[a] *Operational Medicine Section, Trauma Group, Defence Research & Development Canada (DRDC) Toronto, 1133 Sheppard Avenue West, Toronto, Ont. M3M 3B9, Canada*

[b] *University of Toronto, The Hospital For Sick Children, Research Institute, Toronto, Ont., Canada*

[c] *NociPharm, Scarborough, Ont., Canada*

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Summary

We assessed the in vitro bactericidal efficacy of a new sunburn gel (Rescuderm™; RESC) against planktonic and sessile *Pseudomonas aeruginosa* (PSEUD) and *Staphylococcus epidermidis* (STAPH). While PSEUD levels were 4 $\log_{10}$ lower than those of STAPH within 24 h of adding RESC to contaminated nutrient broths, all bacterial counts were comparable by 48 h. PSEUD and STAPH levels were then measured after applying either a single or three consecutive aliquots of RESC to polyurethane sponges. Gel was removed after 5 or 20 min, or left on for 72 h. Bacterial counts in placebo-treated sponges had plateaued by 24 h to values above 9 $\log_{10}$ CFU/mL. In contrast, six out of seven of the RESC application modalities reduced bacterial levels below 4 $\log_{10}$ CFU/mL for 72 h. RESC remained effective against STAPH despite up to a 24 h treatment delay, irrespective of the number of applications. Repeated RESC applications were required to maintain PSEUD below 4 $\log_{10}$ CFU/mL when the delay exceeded 7 h. These data demonstrate the differential susceptibility of planktonic and sessile bacteria to RescuDerm™. This product might be a good candidate for reducing the opportunity for wound infection, especially in burns.

Keywords: Burns; Amorphous gel; Biofilms

1. Introduction

Infection remains a serious complication in thermally injured patients, with *Pseudomonas aeruginosa* being the most abundant and dangerous pathogen [1]. However, the incidence of *Staphylococcus epidermidis* infections has increased in recent years, due to their prevalence on human skin, their ability to adhere to biomaterials, and a greater resistance to most antibiotics [2]. While early excision of full-thickness injuries and closure of the burn wound have been shown to markedly decrease the incidence of infection for burns covering less than 15% of the total body surface area, the risk of wound sepsis remains high, particularly for larger burns [3,4]. Various topical antibacterial therapies have shown clinical effectiveness in the prevention and control of burn infections, with 1% silver sulfadiazine creams and 5% mafenide acetate solutions being the most commonly used topical preparations [1,5]. However, all current antimicrobial agents exert potentially detrimental side effects, such as allergic reactions, emergence of resistant strains of micro-organisms, and delays in wound healing [6]. Thus, the search for the ideal topical agent continues.

Notwithstanding the risk of infection, burn injuries have the additional requirement that they should be cooled soon after wounding to reduce the severity of tissue damage, decrease initial local oedema, and improve wound healing [7–9]. Several studies have reported that amorphous gels and hydrogel sheet dressings provide a moist environment that promotes initial cooling and subsequent healing of burn wounds [8,10,11]. Due to their high water content, hydrogels are also typically effective drug delivery systems for the treatment of contaminated wounds, including burns [12,13].

Rescuderm™ (NociPharm Inc., Scarborough, Ont.), an amorphous gel containing acetic acid, citric acid, and ethylenediaminetetraacetic acid, is currently recommended for its soothing properties when applied on sun- and heat

* Corresponding author. Tel.: +1 416 635 2165; fax: +1 416 635 2132.
E-mail address: lucie.martineau@drdc-rddc.gc.ca (L. Martineau).

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burns. The product, patented for its analgesic and wound healing properties [14], anecdotally appears to have bactericidal function. The unique combination of these properties, if confirmed experimentally, would make this product a most valuable strategy for the management of burn wounds. This study was undertaken to assess the in vitro bactericidal efficacy of Rescuderm™ against planktonic and sessile *Ps. aeruginosa* and *Staph. epidermidis*.

2. Materials and methods

Aliquots of RescuDerm™ (RESC) and placebo gel (PLAC) were prepared under aseptic conditions by the manufacturer; packaged individually in sterile tubes. Assessment of the sterility of the different aliquots was performed prior to their use by: immersing random samples of the gels in sterile Trypticase Soy broth; incubating the aliquots overnight at 37 °C; plating serial dilutions on Tryptic Soy agar enriched with 5% sheep blood (PML Microbiologics, Mississauga, Ont.) to assess the presence of bacteria in the nutrient broth.

2.1. Assessment of bactericidal efficacy against planktonic bacteria

Ps. aeruginosa- (ATCC 27317; PSEUD) and *Staph. epidermidis*-coated beads (ATCC 12228; STAPH) were placed in 20 mL of sterile Trypticase soy broth and Brain Heart Infusion broth, respectively (VWR, Mississauga, Ont.). Bacterial cultures were incubated at 37 °C for 16 h. Cultures were then centrifuged (2500 rpm; 20 min; 4 °C), and the pellets were washed three times with phosphate buffered saline (PBS). The final concentration of the preparation was assessed by standard colony counts on Trypticase soy agar enriched with 5% sheep blood. Fresh Trypticase soy broth (for PSEUD) or Brain Heart Infusion broth (for STAPH) was added to tubes containing the washed bacterial inoculum to obtain an approximate concentration of 10^4 colony-forming units (CFU) per mL. A 200 μL aliquot of either PLAC or RESC ($n = 6$ per group) was then added to 3 mL of the bacterial suspension. The mixture was then vortexed until complete dissolution of the gel and incubated at 37 °C for up to 48 h. A standard plate count was performed in duplicate after 4, 6, 24 and 48 h to assess the presence of propagation-competent PSEUD and STAPH in the gel-supplemented broths.

2.2. Assessment of bactericidal efficacy against sessile bacteria

The bactericidal efficacy of RESC against sessile Gram-negative and Gram-positive bacteria was assessed using a modification of Grzybowski's in vitro ''germ carrier'' model [15]. Briefly, washed PSEUD and STAPH bacterial cultures were prepared as described previously. A mixture of Mueller-Hinton broth and fetal calf serum (50:50) was added to serial tubes containing the bacterial inoculum to obtain a minimum concentration of 10^2 colony-forming units (CFU) in 750 μL. A 1 cm^2 piece of sterile polyurethane sponge (Hydrasorb™; Avitar, Canton, MA) was then placed in the diluted inoculum for 5 min for complete uptake of the bacterial inoculum. The PSEUD- and STAPH-seeded sponges ($n = 6$ per strain per experimental condition) were then transferred individually onto sterile Nalgene caps (VWR, Mississauga, Ont.) in a 15 cm Petri dish containing sterile distilled water (5 mm deep).

Different application modalities of RESC were used to assess the bactericidal efficacy of the product. Firstly, a single 200-μL aliquot of either PLAC or RESC was spread over each sponge immediately after their seeding, and the gel remaining at the surface was gently wiped off after 5 or 20 min, or left on for 72 h. This protocol simulated a scenario where a wound was superficially contaminated and immediately treated. Alternately, a single aliquot of PLAC or RESC was applied 4, 7 or 24 h after completion of the seeding procedures, the gels remaining on the surface of the sponges after their application for the entire 72-h study. This protocol simulated a battlefield scenario where treatment was delayed, whereby the wound contamination progressed to infection before any medical intervention took place. Lastly, the gel was applied using the original manufacturer's suggestions, spreading 200-μL aliquots of either PLAC or RESC over PSEUD- or STAPH-seeded sponges, and wiping them off after 10 min. This cycle was repeated twice, and a third application of RESC was then left on the sponges for up to 72 h. The manufacturer had previously determined that this procedure was optimal for the full expression of the analgesic properties of RESC (unpublished data). The bactericidal efficacy of a 5% mafenide acetate gel prepared using PLAC was also assessed as a positive control using the immediate (i.e., left on for 72 h) and 24-h delayed application protocols.

The Petri dish was then covered to maintain the gel-covered sponges moist, and incubated at 37 °C for various time intervals. The sponges were then aseptically placed into tubes containing 3 mL of an aqueous solution of 1% Tween 80, and rocked on an orbital shaker (400 rpm; 7 min; 4 °C). The solution was expressed from the sponges that were transferred to another Tween 80-containing tube and rocked as per the previous step. Following removal of the Tween mixture, the sponges were immersed into 4 mL of PBS, and sonicated at 4 °C for 45 s. A standard plate count was performed in duplicate using the pooled Tween 80 solutions to assess the presence of PSEUD and STAPH at various time intervals following the seeding of the sponges.

2.3. Statistical analysis

Statistical analyses were completed using Statistica (Version 6.1, Statsoft, Inc.). In all studies, a two-way analysis of variance (ANOVA) with two within-subject variables (time elapsed since application of gel; type of gel)

was used to determine statistical significance among groups for respective differences in bactericidal efficacy. When statistical significance was determined for main or interaction effects, a Neumann–Keuls post hoc analysis was performed to locate significant differences. Significance was deemed to exist when $p < 0.05$.

3. Results

3.1. Assessment of bactericidal efficacy against planktonic bacteria

Fig. 1 depicts the changes in bacterial counts over 48 h following the addition of a single aliquot of RESC or the negative control gel (i.e., PLAC) into nutrient broth seeded with either *Ps. aeruginosa* or *Staph. epidermidis*. PSEUD and STAPH bacterial counts in the PLAC-treated broth increased to 8.98 ± 0.07 and 8.15 ± 0.05 $\log_{10}$ CFU/mL within 24 h, respectively ($p < 0.001$). While PSEUD levels plateaued for the next 24 h, a small but significant reduction (7%; $p < 0.05$) in STAPH counts was observed over that period. Furthermore, STAPH levels in PLAC-treated broth remained significantly lower ($p < 0.001$) than PSEUD counts throughout the 48-h study.

Addition of a single aliquot of RESC to the nutrient broths significantly ($p < 0.01$) reduced both PSEUD and STAPH counts within 4 h of incubation compared to counts in PLAC-treated broth, the bactericidal efficacy of RESC against the two different bacteria being comparable for the first 6 h. PSEUD levels in RESC-treated broth had decreased ($p < 0.001$) to 0.63 ± 0.63 $\log_{10}$ CFU/mL within 24 h, these levels plateauing for the next 24 h. While the RESC-induced reduction in STAPH counts after 24 h was 4 $\log_{10}$ smaller ($p < 0.05$) than that observed for PSEUD, similar low counts were measured by 48 h.

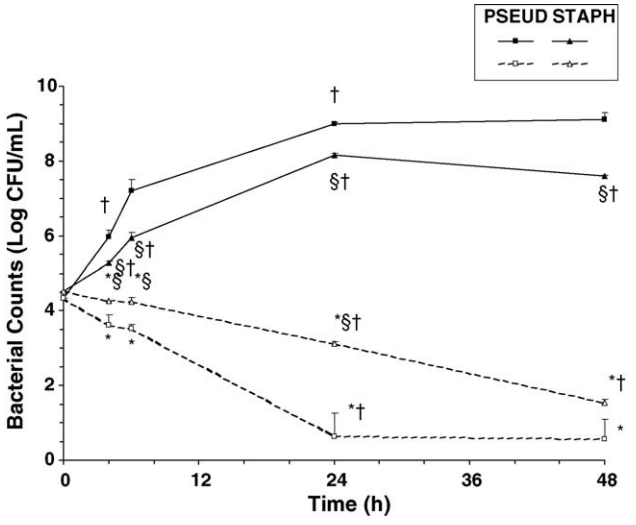

Fig. 1. Bactericidal efficacy of a single aliquot of a placebo gel (filled symbols) or RescuDerm™ (open symbols) placed into nutrient broth seeded with either *Ps. aeruginosa* (PSEUD) or *Staph. epidermidis* (STAPH). Data are expressed as mean ± standard error of the mean (S.E.M.; $n = 6$). Dashed lines indicate different from placebo ($p < 0.05$). *Different from pre-treatment value ($p < 0.05$). †Different from previous time interval. §Different from PSEUD ($p < 0.05$).

3.2. Assessment of bactericidal efficacy against sessile bacteria

Fig. 2 depicts the changes in bacterial counts following a single aliquot of PLAC or RESC applied to the polyurethane sponges immediately after their seeding. PSEUD and STAPH counts in the control placebo-treated sponges increased to 10.02 ± 0.08 and 9.34 ± 0.08 $\log_{10}$ CFU/mL within 24 h, respectively ($p < 0.001$). While PSEUD levels plateaued for the next 48 h, a small reduction (3%; $p < 0.05$) in STAPH counts was observed over that period. Overall, STAPH levels in the PLAC-treated sponges remained significantly lower ($p < 0.001$) than PSEUD counts over the 72-h study.

A 5-min application of a single aliquot of RESC on the PSEUD- and STAPH-seeded sponges eradicated growth and

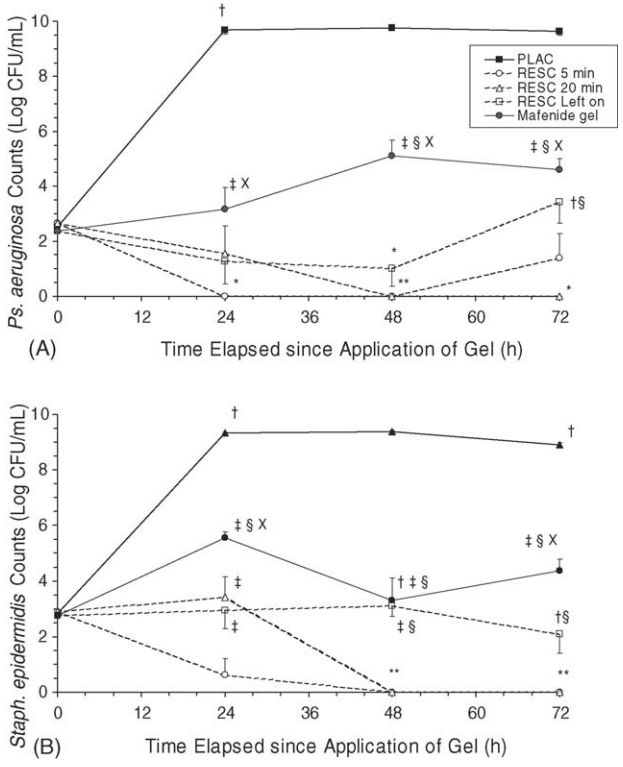

Fig. 2. Changes in bacterial counts following application of a single aliquot of a placebo gel (PLAC; filled symbols), RescuDerm™ (RESC; open symbols), or 5% mafenide acetate gel on polyurethane sponges immediately after their seeding with either *Ps. aeruginosa* (Panel A) or *Staph. epidermidis* (Panel B). Gels were removed after either 5 or 20 min (PLAC and RESC only), or remained on the sponges for 72 h. Data are expressed as mean ± S.E.M. ($n = 6$). Dashed lines indicate different from PLAC ($p < 0.05$). *Different from pre-treatment value ($p < 0.05$). †Different from previous time interval. ‡Different from $RESC_{5\text{-min}}$. §Different from $RESC_{20\text{-min}}$. ×Different from $RESC_{left\ on}$.

biofilm formation after 24 and 48 h, respectively ($p < 0.01$). While PSEUD counts were comparable to pre-treatment values after 72 h (1.40 ± 0.89 $\log_{10}$ CFU/mL), this level of contamination represented an approximately 8-$\log_{10}$ reduction compared to that of placebo-treated sponges (Fig. 2A). Increasing the duration of application of RESC to 20 min had completely eradicated ($p < 0.001$) both PSEUD and STAPH biofilm formation after 48 h, no bacterial growth being measured in any of the previously seeded sponges for the remainder of the 72-h study (Fig. 2). Leaving the RESC aliquot on the seeded sponges for 72 h totally prevented the increase in biofilm formation observed for placebo-treated sponges, bacterial counts averaging 3.44 ± 0.78 and 2.09 ± 0.68 $\log_{10}$ CFU/mL after 72 h for PSEUD and STAPH, respectively ($p < 0.001$). The reductions in STAPH and PSEUD levels relative to those of PLAC were comparable throughout the 72-h study following either a 5- or 20-min application of RESC, averaging 8.98 ± 0.19 $\log_{10}$. In contrast, the magnitude of this reduction was greater ($p < 0.05$) for PSEUD than STAPH during the first 48 h of a 72-h application of RESC on the sponges (i.e., 8.81 ± 0.62 $\log_{10}$ versus 6.23 ± 0.40 $\log_{10}$ reduction). RESC was more effective against both PSEUD and STAPH than the 5% mafenide acetate gel, regardless of its application protocol ($p < 0.01$).

Fig. 3 depicts the bactericidal efficacy of RESC under the different application-delay conditions. PSEUD levels rose from 2.38 ± 0.05 to 4.37 ± 0.88 $\log_{10}$ CFU/mL within 4 h of seeding ($p < 0.05$), further increasing to 6.60 ± 0.04 and 9.32 ± 0.05 $\log_{10}$ CFU/mL after 7 and 24 h, respectively ($p < 0.001$; Fig. 3A). While little STAPH growth occurred in the first 4 h after seeding (i.e., time 0 for Figs. 2B versus 3B), these levels had increased to 4.20 ± 0.16 and 9.22 ± 0.07 $\log_{10}$ CFU/mL within 7 and 24 h of seeding, respectively (Fig. 3B). Bacteria counts averaged 9.51 ± 0.06 $\log_{10}$ CFU/mL within 24 h of PLAC application, regardless of the duration of the delay before application of the gel or the strain of bacteria, these levels remaining constant over the next 48 h.

$PSEUD_{4\text{-h delay}}$ levels decreased to 1.10 ± 0.69 $\log_{10}$ CFU/mL within 24 h of applying RESC ($p < 0.05$). These counts were approximately 3 $\log_{10}$ lower than those observed for the 7-h delay condition over that period ($p < 0.05$). However, $PSEUD_{4\text{-h delay}}$ and $PSEUD_{7\text{-h delay}}$ levels were comparable for the remainder of the experiment, averaging 1.23 ± 0.31 $\log_{10}$ CFU/mL. In contrast, $STAPH_{4\text{-h delay}}$ and $STAPH_{7\text{-h delay}}$ counts in RESC-treated sponges remained constant throughout the experiment, and were approximately 5 $\log_{10}$ lower than those of PLAC after 72 h ($p < 0.001$; Fig. 3B). There was a 9-$\log_{10}$ reduction ($p < 0.001$) in PSEUD levels at 72 h in RESC- compared to PLAC-treated sponges when delaying the gel application by up to 7 h. Interestingly, the bactericidal efficacy of RESC against PSEUD was approximately 2.5 $\log_{10}$ greater ($p < 0.05$) when the initial bacterial load was increased from 2.38 ± 0.05 to 6.60 ± 0.04 $\log_{10}$ CFU/mL (Figs. 2A versus 3A).

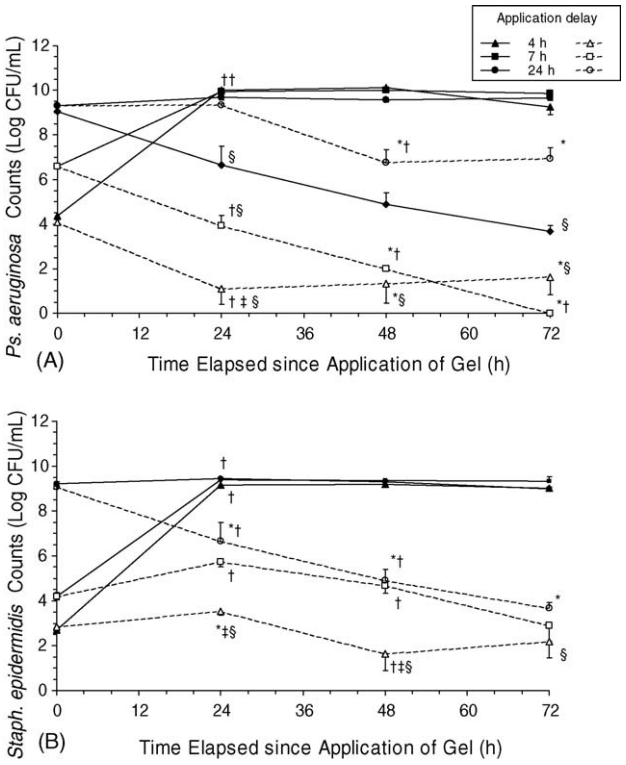

Fig. 3. Changes in bacterial counts following delayed application of a single aliquot of a placebo gel (filled symbols) RescuDerm™ (open symbols), or mafenide acetate gel (diamonds) on polyurethane sponges seeded with either *Ps. aeruginosa* (Panel A) or *Staph. epidermidis* (Panel B). Gel was applied 4, 7, or 24 h after seeding the sponges. Data are expressed as mean ± S.E.M. ($n = 6$). Dashed lines indicate different from placebo ($p < 0.05$). †Different from previous time interval ($p < 0.05$). *Different from pre-treatment value. ‡Different from 7-h delay ($p < 0.05$). §Different from 24-h delay.

While $PSEUD_{24\text{-h delay}}$ levels remained unchanged in the 24 h following RESC application on the seeded sponges, they decreased to 6.95 ± 0.48 $\log_{10}$ CFU/mL within 48 h. $STAPH_{24\text{-h delay}}$ counts decreased to 6.67 ± 0.83 $\log_{10}$ CFU/mL within 24 h ($p < 0.001$), decreasing to $STAPH_{4\text{-h delay}}$ and $STAPH_{7\text{-h delay}}$ values by 72 h. The 5% mafenide acetate gel was more effective than RESC against PSEUD when its application was delayed by 24 h ($p < 0.05$; Fig. 3A). Technical difficulties prevented the assessment of the efficacy of mafenide acetate against STAPH under those conditions.

Fig. 4 depicts the changes in bacterial counts following immediate (IMM MANUF) or delayed (24-h delay MANUF) application of PLAC or RESC on seeded polyurethane sponges using the manufacturer's suggested protocol. PSEUD and STAPH counts in PLAC-treated sponges plateaued within 24 h of application of the gel to approximately 9 $\log_{10}$ CFU/mL, regardless of the duration of the delay or strain of bacteria. $RESC_{IMM\ MANUF}$ reduced PSEUD levels by 8 $\log_{10}$ within 48 h ($p < 0.05$) compared to PLAC, these levels averaging 1.04 ± 0.67 $\log_{10}$ CFU/mL for the remainder of the 72-h study. In contrast, STAPH levels increased ($p < 0.01$) from 2.11 ± 0.01 to 4.03 ± 0.17 $\log_{10}$ CFU/mL within 72 h using $RESC_{IMM\ MANUF}$,

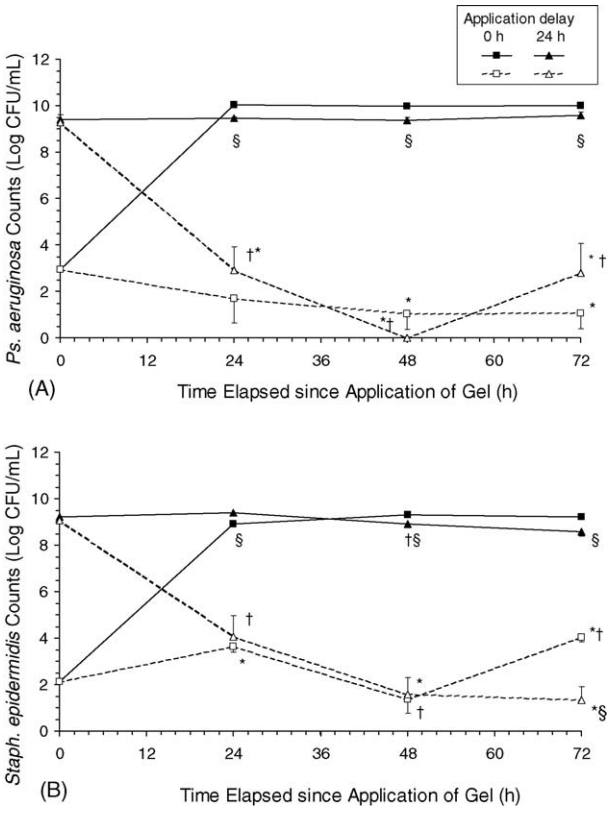

Fig. 4. Changes in bacterial counts following three applications of placebo gel (filled symbols) or RescuDerm™ (open symbols) on polyurethane sponges seeded with either *Ps. aeruginosa* (Panel A) or *Staph. epidermidis* (Panel B). Gel was applied immediately after seeding the sponges or after 24 h, using the manufacturer's suggested protocol (see text for details). Data are expressed as mean ± S.E.M. ($n = 6$). Dashed lines indicate different from placebo ($p < 0.05$). *Different from pre-treatment value. †Different from previous time interval ($p < 0.05$). §Different from immediate application ($p < 0.05$).

compared to approximately 9 $\log_{10}$ CFU/mL in the PLAC-treated sponges. Within 48 h of $RESC_{24\text{-h delay MANUF}}$, PSEUD and STAPH counts decreased ($p < 0.001$) by 9.26 ± 0.05 and 6.04 ± 1.37 $\log_{10}$, respectively ($p < 0.05$). While STAPH levels remained constant for the next 24 h (Fig. 4B), PSEUD levels increased to values comparable to those observed after 24 h of $RESC_{24\text{-h delay MANUF}}$ (Fig. 4A).

4. Discussion

Open wounds are prime targets for bacterial invasion. Thus, topical antibacterial agents play an important role in the management of wounds, especially burns as they can be compounded by marked immunosuppression [16]. Considering the wide variety of microorganisms that have been isolated from burns and the steady emergence of resistant bacterial strains [17], the search for a topical therapy that provides a broad-spectrum antimicrobial activity retains high priority.

The aim of the present study was to identify a potential role for RescuDerm™ as an infection control agent. Our data clearly demonstrate the in vitro bacteriostatic and bactericidal efficacy of this product, regardless of the frequency of its application, the bacterial strain tested, or the initial bioburden. While we observed a differential susceptibility of Gram-negative and Gram-positive bacteria to RescuDerm™, all bacterial levels were maintained well-below 5 $\log_{10}$ CFU, the threshold for clinical infection that impacts on the outcome of wound healing and grafting procedures [18,19]. Our observation of rapid elimination of planktonic bacteria likely contributed to the magnitude of this bactericidal effect, as the bacterial biofilms formed on biological surfaces gain resistance to eradication within as little as 2 h [20]. Our findings, taken together with the observation that RescuDerm™ possesses a significant in vitro bactericidal activity against other pathogen species such as *Streptococcus*, *Salmonella*, *Candida*, and *Listeria* (Prof. Griffiths, Canadian Institute of Food Safety Research, University of Guelph, Ont., Canada; pers. commun.), suggest the potential usefulness of this product for reducing the opportunity for polymicrobial wound infection.

In vitro models of infection such as the microbroth dilution test and contaminated synthetic germ carrier system used in the present study provide effective models for rapid screening of bactericidal and bacteriostatic agents, formulations, and dressings [15,21,22]. However, most in vitro tests are imprecise indicators of in vivo efficacy of the antibacterial agents, as they poorly represent the complex environment at the wound surface, the debris-organism interface, pro-inflammatory reactions and bacterial defense pathways for survival in abnormal environments such as the wounds [23,24]. Hershberger et al. [25] have shown that the fluoroquinolone-induced reductions in *Staphylococcus aureus* and *Enterococcus* sp. levels in an in vitro model of simulated endocarditis were comparable to those observed in a rabbit model of the disease. In contrast, we recently observed comparable in vivo bactericidal efficacies of chlorhexidine- and chloramphenicol-loaded DRDC wound dressings against *Ps. aeruginosa* despite significantly better in vitro performance of chloramphenicol [13]. In a murine model of neutropenic thigh infection, the bactericidal efficacy of ticarcillin against *Ps. aeruginosa* was greater than that observed in an in vitro pharmacodynamic model, partly due to intrinsic differences between in vitro and in vivo bacterial growth rates [26]. The nutrient broth used in our present model system contained several compounds that may have affected the bactericidal efficacy of RescuDerm™. While the addition of serum to brain heart infusion broth seeded with *Salmonella* sp. had no effect on the bactericidal activity of acetic acid [27], Entani et al. [28] reported that the combination of vinegar and sodium chloride had a synergistic effect on the intrinsic bactericidal activity of acetic acid, the latter being one ingredient of RescuDerm™, which also contains sodium chloride as a result of pH adjustment with NaOH. Studies are underway to determine the magnitude of RescuDerm's bactericidal properties in full-thickness wounds carrying different bioburdens.

One important attribute of topical agents is their ability to penetrate the wounds and destroy pathogens that might have invaded the underlying damaged and healthy tissues. In this

respect, water-soluble solutions of mafenide acetate appear more bactericidal than cream preparations when applied on burn eschars [29,30]. In preliminary studies, we determined gravimetrically that approximately 60% of the volume of RescuDerm™ gel applied on the hydrophilic polyurethane sponges is absorbed within 20 min (unpublished data). In contrast, the corresponding value amounted to less than 4% when the product was applied on either excised rat skin or skeletal muscle tissue. These preliminary measurements did not examine the likely hydrogen ion flux along the gel–tissue and gel–sponge gradient. Herruzo-Cabrera et al. [31] reported complete eradication of the bacterial loads in artificial pig eschars treated for 3 days with various topical agents, whose penetration fractions ranged from 0.3 to 0.8%. Clearly, complex factors including host responses, timing of the application relative to the initial bioburden, the virulence and mix of invading pathogens, as well as the mechanism of action of the topical antibacterial agents will combine to determine the net in vivo efficacy.

Management of wounds sustained by military casualties offers additional challenges to those encountered when treating comparable civilian wounds. Besides wound size, severity of injury, increased risk of contaminations and re-contaminations, military wound care may not only be delayed, but may of necessity be self-administered or facilitated by untrained personnel in extremely challenging and probably hostile environments [32]. These factors exacerbate wound infection and the risk of systemic infection. These tactical scenarios, and the potential for high casualty incidences that challenge infrastructures, have driven the search for an efficacious, field-usable wound care system. Based on our experimental results, it appears that the application of a single dose of RescuDerm™ to moderately contaminated wounds would prevent further bacterial growth or even significantly reduce their bioburden within the critical, first post-trauma day. Interestingly, this amorphous gel was more bactericidal than a 5% mafenide acetate solution, the latter being recommended for the treatment of both burned casualties evacuated from the battlefield and civilians [6,30]. While the bactericidal efficacy of mafenide acetate became superior to that of RescuDerm™ when the initial bacterial loads were comparable to those of abscesses or chronically infected wounds, increasing the frequency of application of the product yielded comparable efficacies. Clearly, the benefit of repeated applications would be practical mainly for smaller wounds in front-line casualties, less so for more extensive wounds that require evacuation under extreme environmental conditions (e.g., open fire, potential exposure to noxious chemicals, darkness, etc.). However, Kauvar et al. [33] have recently shown that immediate application of solutions of either 5% mafenide acetate or 4% chlorhexidine digluconate to non-debrided thermal injuries contaminated with a virulent strain of *Ps. aeruginosa* failed to prevent systemic infection and mortality. Mafenide acetate and silver sulfadiazine creams are deemed too cumbersome for use in combat zones [30,33], and neither provides RescuDerm's analgesia, associated with the transient blockade of vanilloid receptor-1 firing in a major nociceptor subset of sensory afferent nerves which generate pain sensation [14]. While relevant pre-clinical antimicrobial data are unavailable, our present findings suggest a possible role for RescuDerm™ for providing early infection prophylaxis in front-line casualties.

Several laboratories have shown that while current topical antimicrobial agents such as silver sulfadiazine and mafenide acetate effectively eradicate bacteria, they may also be harmful to many of the cells involved in wound healing at the concentrations used clinically [34,35]. To this effect, in vitro studies have shown that application of a 0.25% (w/w) acetic acid solution has profound cytotoxic effects on human keratinocyte and fibroblast growth [36,37], this concentration being lower than that in the RescuDerm™ formulation tested in the present studies. However, complete healing of a postoperative abdominal midline wound infected with *Ps. aeruginosa* was observed following irrigation with 3% acetic acid once daily for 10 days [38]. Sloss et al. [39] have also shown that short-term application of 5% acetic acid soaks twice-daily effectively eradicated *Ps. aeruginosa* from burns and other soft tissue wounds, and led to wound healing. Clearly, the modality of application of RescuDerm™ (e.g., frequency and duration of application) that will optimize its bactericidal effect while allowing wound healing to proceed uneventfully must be established for a variety of wounds, including burns.

These data show that RescuDerm™, an amorphous water-soluble gel currently recommended for its soothing properties on sun- and heat-burns, is an effective bactericidal agent against common planktonic and sessile Gram-negative and Gram-positive pathogens. This property, taken together with potential advantages such as low cost, ease of application and removal by rinsing with saline, negligible risk of allergic reactions and limited antimicrobial resistance due to the nature of its ingredients, suggest considerable value of this gel formulation for treating wounds, especially burns.

Potential conflict of interest

This work was partly supported by NociPharm Inc. DRDC Toronto designed all experimental protocols. The findings of this study were not constrained by the sponsoring body.

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