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Biofilm eradication by a new burn gel that targets nociception.

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SUMMARY

RescuDerm™ (RESC) is an amorphous water-soluble gel containing vinegar, citric acid, and EDTA. It targets TRPV1+ dermal sensory afferent nerves (nociceptors) and is patented for its soothing properties in superficial burns. We recently discovered effective bactericidal activity in RESC, against planktonic Gram-negative and Gram-positive pathogens. Bacterial biofilms remain a clinically challenging event in wound infection. We compared the ability of RESC and various derivative formulations to eradicate *Ps. aeruginosa* (PSEUD) and *Staph. epidermidis* (STAPH) biofilms within 4-24 hr exposures in MBEC devices. Citric acid-free, acetic acid-free or acetic acid-free/sodium acetate-supplemented RESC gels reduced PSEUD and STAPH biofilm formation as effectively as RESC. Substituting the weak organic acids with equivalent concentrations of glacial acetic acid reduced the gel's effectiveness against PSEUD and STAPH biofilms by half, but viable bacterial counts still remained below 4 $\log_{10}$ CFU/peg. Removal of gelling agent and/or EDTA enhanced efficacy against PSEUD but not STAPH biofilms. An acidified placebo gel formulation generated an only marginal bactericidal effect compared to that of RESC. RESC is a promising new antimicrobial agent, its weak organic acid content, rather than merely acidic pH mediating its considerable *in vitro* bactericidal efficacy against bacterial biofilms.

Keywords: Acetic acid; citric acid; carbopol; EDTA; pH; Minimum Biofilm Elimination Concentration (MBEC™) Assay System; Transient Receptor Potential Vannelloid-1 (TRPV1) positive nociceptive nerves.

1. Introduction

Systemic sepsis resulting from invasive infection remains the leading cause of death among thermally-injured patients. Burn wounds are a major focus for infection, as they become readily colonized with several species of potentially pathogenic microorganisms, including *Pseudomonas aeruginosa* and *Staphylococcus sp* [1]. For example, *Staphylococcus epidermidis* infections have been reported not only in burn wounds but also in skin grafts and donor sites, likely due to the prevalence of this microorganism on human skin and its resistance to most antibiotics [2-5]. Various topical antibacterial therapies have shown clinical effectiveness in the prevention and control of infection in burns, skin-graft sites, and donor sites, with 1% silver sulfadiazine creams and 5% mafenide acetate solutions being the most commonly used topical preparations [1, 6-8]. However, all current antimicrobial agents can mediate potentially detrimental side effects, including allergic reactions, emergence of resistant strains of micro-organisms, and delays in wound healing [9]. Furthermore, there is consensus that biofilms are harder to eradicate than their planktonic counterparts, as sessile bacteria more effectively resist adverse environments by forming aggregates, adapting phenotypes, and/or generating metabolic changes to evade hostile milieu and host immune responses [10-11]. Evidence supporting the presence of biofilms on the surface of chronic human wounds [10] as well as in animal models of acute partial-thickness wounds [12] and burns [13] has been reported. Thus, the use of suitable broad-spectrum topical agents that inhibit biofilm formation or promote their detachment should be integral to the management of wound infection.

Rescuderm^{TM} (NociPharm Inc., Toronto, Ontario, Canada) is an amorphous,

organic acid-based gel recommended for its soothing properties in sun-, heat and X-ray burns, due to its transient blockade of dermal TRPV1+ nociceptors and their neurogenic, proinflammatory and pain generating effects [14]. Patented for its analgesic and anti-inflammatory properties [15], Rescuderm^{TM} might also be a candidate for reducing opportunistic wound infection, since recent *in vitro* studies using synthetic polyurethane germ carriers revealed that a range of Rescuderm^{TM} application modalities either effectively eradicated planktonic and adherent populations of *Ps. aeruginosa* and *Staph. epidermidis* or maintained their levels at 2-4 $\log_{10}$ CFU/mL [16]. However, it remains unclear whether our previous model system allowed the formation of mature biofilms or only the irreversible attachment of the bacteria to the substrate. The aims of the present report were to assess the *in vitro* efficacy of Rescuderm^{TM} against *Ps. aeruginosa* and *Staph. epidermidis* biofilms, and to identify which ingredient or combination thereof confers this activity.

2. Materials and methods

Rescuderm^{TM} is an amorphous gel containing organic acids (acetic acid, 1% organic vinegar; citric acid, 4%), chelating and gelling agents (disodium EDTA and Carbopol® 940, respectively), and deionized water in proprietary concentrations. Nine formulations of Rescuderm^{TM} (RESC) with various pH, viscosities, and compositions were compared in the present study (Table 1). Aliquots of modified RESC and placebo (water) gel (PLAC without active ingredients) were prepared under aseptic conditions by the manufacturer, and packaged individually in sterile tubes, adjusting the pH of gel formulations with sodium hydroxide as required. Sterility of the different aliquots was confirmed prior to their use by: immersing random samples of the gels in sterile Trypticase Soy broth; incubating the aliquots overnight at 37^{o}C; and, plating serial

dilutions on Tryptic Soy agar enriched with 5% sheep blood (PML Microbiologics, Mississauga, Ontario, Canada) to assess the presence of bacteria in the nutrient broth.

2.1. Preparation of bacterial inocula

Ps. aeruginosa- (ATCC 27317; PSEUD) and *Staph. epidermidis*-coated beads (ATCC 12228; STAPH) were placed in 20 mL sterile Trypticase Soy broth or Brain Heart Infusion broth, respectively (VWR, Mississauga, Ontario, Canada). Bacterial cultures were incubated at 37˚C for 16 hr, centrifuged (2500 rpm; 20 min; 4˚C), and pellets washed 3x with Phosphate Buffered Saline (PBS). Bacterial counts of the washed inoculum were assessed by standard plate counts on Trypticase Soy agar enriched with 5% sheep blood. Bacterial inocula were then diluted with either fresh Trypticase Soy broth (PSEUD) or Brain Heart Infusion broth (STAPH) to an approximate concentration of 7 $\log_{10}$ CFU per mL.

2.2. Assessment of bactericidal efficacy against biofilms

We assessed the bactericidal efficacy of the gel formulations using the Minimum Biofilm Elimination Concentration (MBEC™) Assay System (MBEC BioProducts, Edmonton, Alberta, Canada; 17). The MBEC™ Assay System consists of a reactor allowing the simultaneous formation of 96 equivalent biofilms from one isolate or biofilms from up to 96 different isolates in a 96-well microtitre plate covered by a lid carrying 96 immersing plastic pegs. Biofilm-laden pegs can then be simultaneously exposed to the same or different treatments. Scanning electron microscopy demonstrated

that maximal growth of test bacteria correlated with the development of mature biofilms [17-21]. The MBEC™ Assay System has proven to be a powerful tool for the screening of aqueous solutions of antibiotics [17, 21-23] and biocides [18-20].

To our knowledge, the MBEC™ Assay System was never used for testing amorphous gels, and we therefore first assessed the suitability of the procedure for our present purpose. Briefly, a 200-μL aliquot of the diluted cultures was added to the test wells of the microtitre plate. The latter was then: covered with the MBEC™ lid, placed on a gyrating platform at approximately 150 rpm and incubated at 37˚C to generate PSEUD and STAPH biofilms of approximately 4-5 $\log_{10}$ CFU/peg. Following overnight incubation, the MBEC™ lid was rinsed for 1 min in a different microtitre plate containing sterile PBS (200 μL per well), to both remove planktonic bacteria from the pegs and growth medium carried over from the growth phase of the assay. Six pegs were then broken from the lid using sterile forceps; transferred to a tube containing 500 μL PBS and sonicated for 5 min to dislodge the biofilms. Serial dilutions of the sonicated bacterial solutions were then plated on Tryptic Soy agar to assess the presence of viable bacteria. Pegs containing PSEUD and STAPH biofilms (n=6 per type of biofilm) were then tested for susceptibility in a challenge plate containing serial dilutions of the placebo gel prepared using either TSB or BHI. Biofilm growth was assessed after 24 hr, as described above. These pilot experiments determined that the minimum dilution factor ensuring maximum recovery of biofilms exposed to PLAC control gel was 1:4 (PSEUD) or 1:8 (STAPH; data not shown). These results suggested that raising the viscosity of culture media beyond a strain-specific threshold reduces the efficiency of sessile growth on MBEC pegs, most likely through shearing of bacteria into liquid phase.

The following procedures were thus used to evaluate the bactericidal activity of the different gel formulations to be tested. PSEUD and STAPH biofilms were prepared as above, and challenged in control broth (Trypticase Soy broth for PSEUD, Brain Heart Infusion for STAPH), or diluted experimental gels (n=6 pegs/gel tested). PSEUD and STAPH biofilms were recovered after either a 4- or 24-hr exposure to the diluted gels, and viable cells in the biofilms were enumerated by performing a standard plate count on Trypticase Soy agar enriched with 5% sheep blood.

2.4. Statistical analysis

Statistical analyses employed Statistica (Version 6.1, Statsoft, Inc.). In all studies, a two-way analysis of variance with two within-subject variables (time elapsed since exposure of biofilm to gel, type of gel) was used to compare differences among groups in bactericidal efficacy. When statistical significance was determined, a Neumann-Keuls *post-hoc* analysis was performed to locate significant differences. Significance was set to 5%, all tests were two-tailed.

3. Results

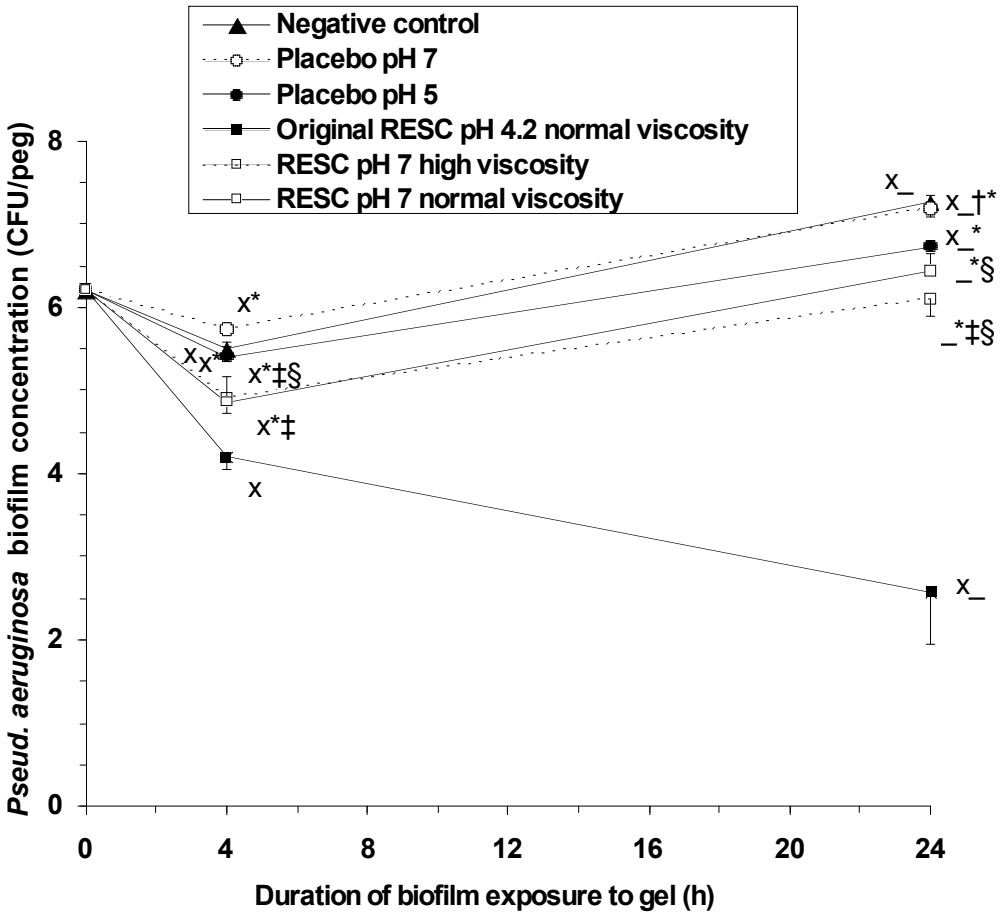
3.1. Role of pH and viscosity

Fig. 1 depicts the effect of alterations in pH and/or viscosity of the original RescuDerm^{TM} formulation on biofilm cell viability. Both PSEUD and STAPH cell viability was reduced ($p<0.05$) within 4 hr of exposing the biofilm-loaded pegs to most of the different experimental gels, while the number of viable bacteria in the different

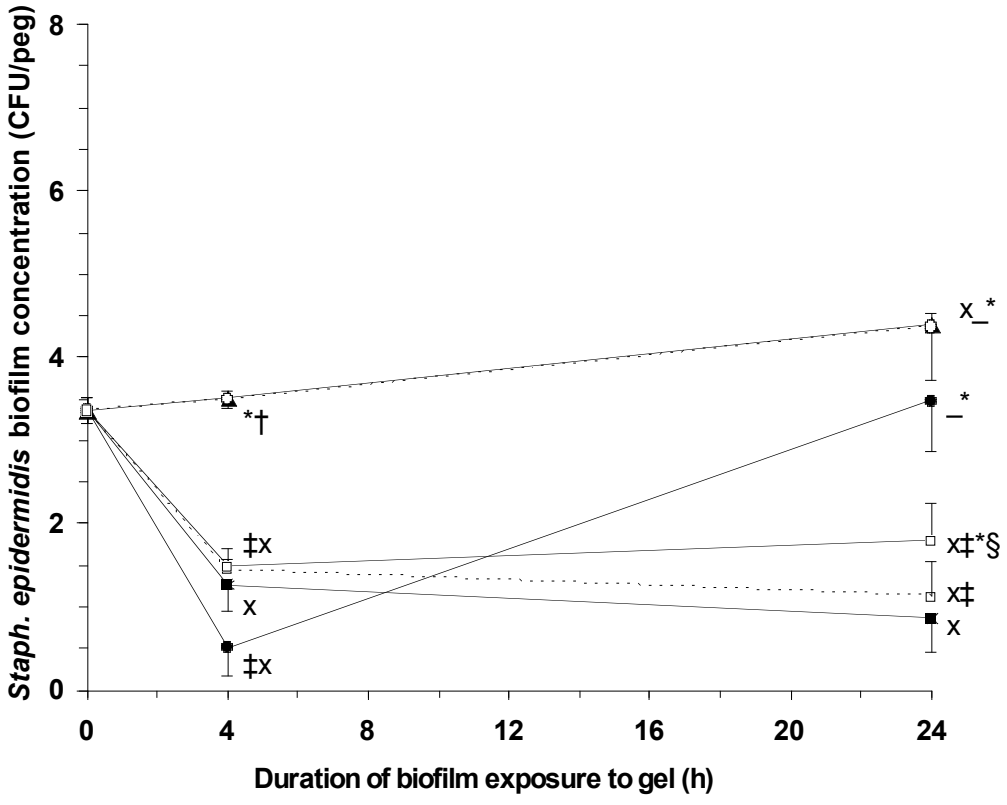
biofilms exposed for 24 hr to the placebo (water) gel was significantly greater than the pre-exposure values ($p<0.05$). The pH of the placebo gel had no significant effect on bacterial viability in STAPH biofilms over a 24-hr period (Fig. 1B). In contrast, a small reduction of viable PSEUD was observed when the biofilms were exposed for 24 hr to the acidic rather than the neutral placebo gel (0.63 ± 0.08 $\log_{10}$ CFU/peg; $p<0.05$). However, these levels still corresponded to an 11% increase above the pre-exposure values ($p<0.05$; Fig. 1A).

Compared to broth or placebo gel controls, exposure of PSEUD and STAPH biofilms to the original RESC formulation reduced the numbers of viable bacteria within 4 hr by 1.30 ± 0.12 $\log_{10}$ and 2.24 ± 0.34 $\log_{10}$ CFU/peg, respectively ($p<0.05$). Prolonging exposure to the original RESC to 24 hr further reduced PSEUD viability to 2.58 ± 0.62 $\log_{10}$ CFU/peg (Fig. 1A; $p<0.05$), while STAPH biofilms contained viable counts that were 1.71 ± 0.66 $\log_{10}$ CFU/peg lower ($p<0.05$) than those of PSEUD. Exposure of PSEUD biofilms for 24 hr to a RESC derivative with neutral pH abolished the bactericidal effect of the original RESC, irrespective of the viscosity of the gel (Fig. 1A). In contrast, more STAPH biofilm bacteria survived ($p<0.05$) upon a 24-hr exposure to a RESC formulation with a neutral pH and a viscosity comparable to that of the original RESC (Fig. 1B). However, irrespective of the viscosity or pH of RESC, the number of viable STAPH sessile bacteria was maintained below 2 $\log_{10}$ CFU/peg.

A.


Negative control
Placebo pH 7
Placebo pH 5
Original RESC pH 4.2 normal viscosity
RESC pH 7 high viscosity
RESC pH 7 normal viscosity
Pseud. aeruginosa biofilm concentration (CFU/peg)
Duration of biofilm exposure to gel (h)
0
2
4
6
8
0
4
8
12
16
20
24
x_
x_†*
x_*
_*§
_*‡§
x*
x*‡§
x*‡
x
x_

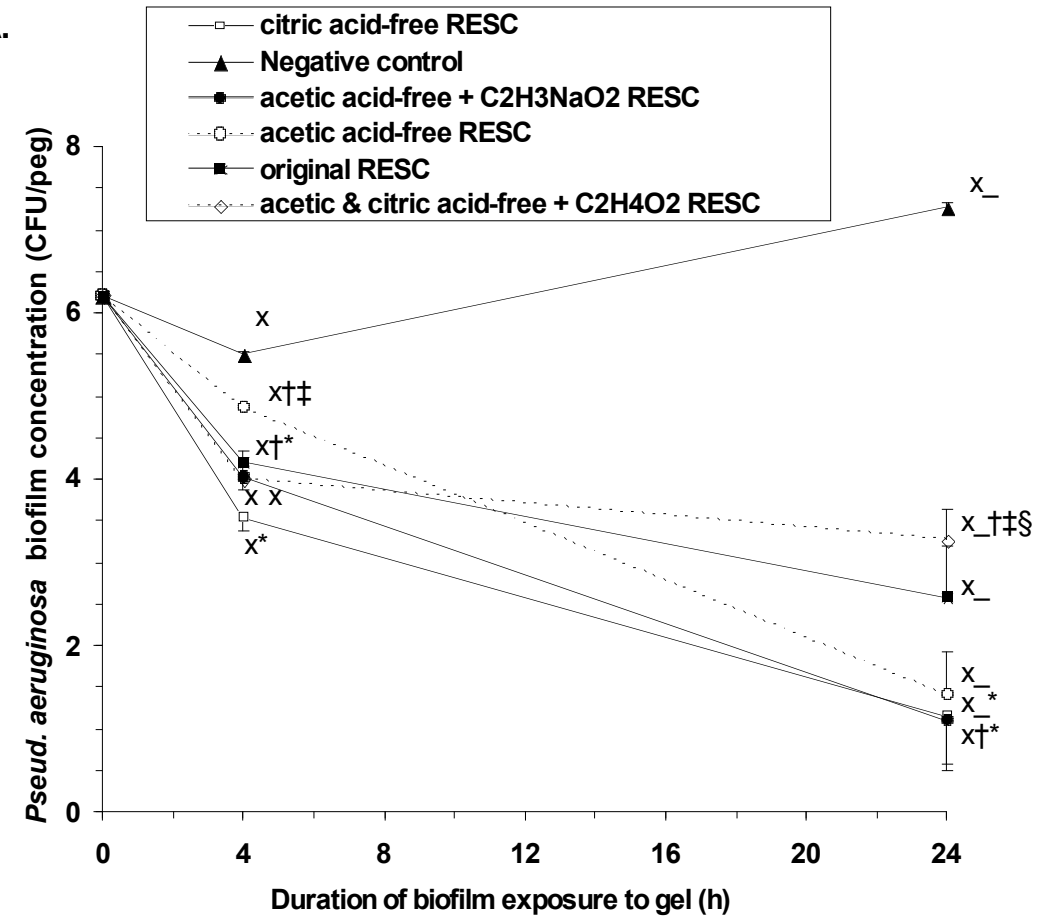
B.


Staph. epidermidis biofilm concentration (CFU/peg)
Duration of biofilm exposure to gel (h)
0
2
4
6
8
0
4
8
12
16
20
24
x_*
*†
_*
‡x
x‡*§
x‡
x
‡x
x

3.2 Role of citric acid and acetic acid

Fig. 2 compares the bactericidal efficacy of acetic acid- and/or citric acid-free gels against PSEUD and STAPH biofilms to that of the original formulation of RESC. PSEUD and STAPH biofilm counts were reduced ($p<0.05$) within 4 hr of exposing the biofilm-loaded pegs to any of the experimental gels. PSEUD and STAPH levels following a 24-hr exposure to an acetic acid-free RESC gel were comparable to those of the original RESC, averaging 1.42 ± 0.51 and 0.83 ± 0.37 $\log_{10}$ CFU/ peg, respectively. PSEUD survival was reduced ($p<0.05$) by approximately 1.5 $\log_{10}$ CFU/ peg compared to that of the original RESC when either sodium acetate was substituted for the acetic acid or citric acid was removed from the gel formulation (Fig. 2A). In contrast, STAPH biofilm survival remained unaffected by these modifications of the original RESC formulation (Fig. 2B). The number of STAPH in biofilms surviving exposure to a gel containing 1% glacial acetic acid instead of the two weak organic acids was significantly increased ($p<0.05$), but viable bacteria levels remained significantly (2 $\log_{10}$ CFU/peg) below those of the controls ($p<0.05$; Fig. 2B). In contrast, the bactericidal efficacy of the glacial acetic acid-containing gel against PSEUD biofilms was comparable to that of the original RESC (Fig. 2A).

A.


citric acid-free RESC
Negative control
acetic acid-free + C2H3NaO2 RESC
acetic acid-free RESC
original RESC
acetic & citric acid-free + C2H4O2 RESC
Pseud. aeruginosa biofilm concentration (CFU/peg)
Duration of biofilm exposure to gel (h)
0
2
4
6
8
0
4
8
12
16
20
24
x_
x
x†‡
x†*
x x
x*
x_†‡§
x_
x_
x_*
x†*

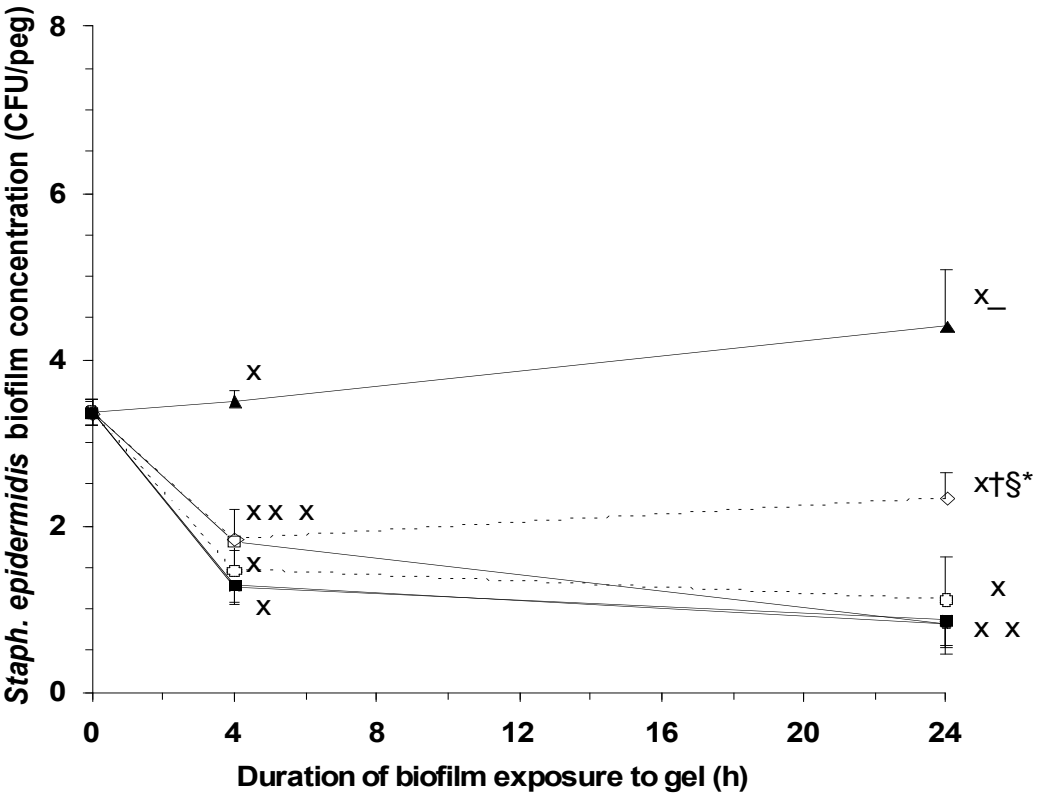
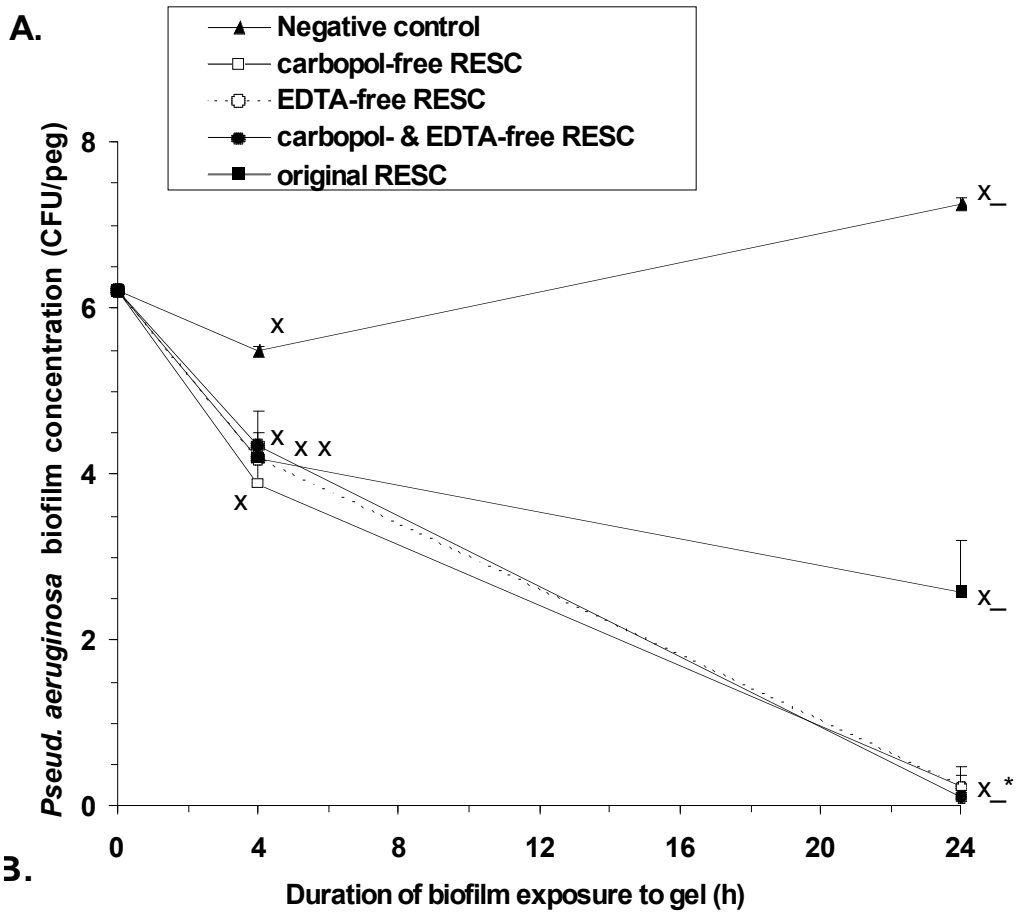

Staph. epidermidis biofilm concentration (CFU/peg)
Duration of biofilm exposure to gel (h)
0
2
4
6
8
0
4
8
12
16
20
24
x_
x
x†§*
x x x
x
x
x
x
x x

Fig. 2

3.3. Role of Carbopol® and Disodium EDTA

Fig. 3 compares the bactericidal efficacy of Carbopol®- and/or EDTA-free gels against PSEUD and STAPH biofilms to that of the original formulation of RESC. PSEUD and STAPH biofilm counts were reduced ($p<0.05$) within 4 hr of exposing the biofilm-loaded pegs to any of the different experimental gels. PSEUD biofilms were nearly eradicated following a 24-hr exposure to RESC formulations that did not contain either Carbopol® and/or EDTA, these bacterial levels being reduced by 2.38± 0.72 $\log_{10}$ compared to those of RESC-exposed biofilms ($p<0.05$; Fig. 3A). In contrast, the susceptibility of the STAPH biofilms to these different RESC gel formulations was comparable to that of the original RESC (Fig. 3B).


A.
Negative control
carbopol-free RESC
EDTA-free RESC
carbopol- & EDTA-free RESC
original RESC
Pseud. aeruginosa biofilm concentration (CFU/peg)
0
2
4
6
8
0
4
8
12
16
20
24
Duration of biofilm exposure to gel (h)
x
x
x x x
x
x_
x_
x_*

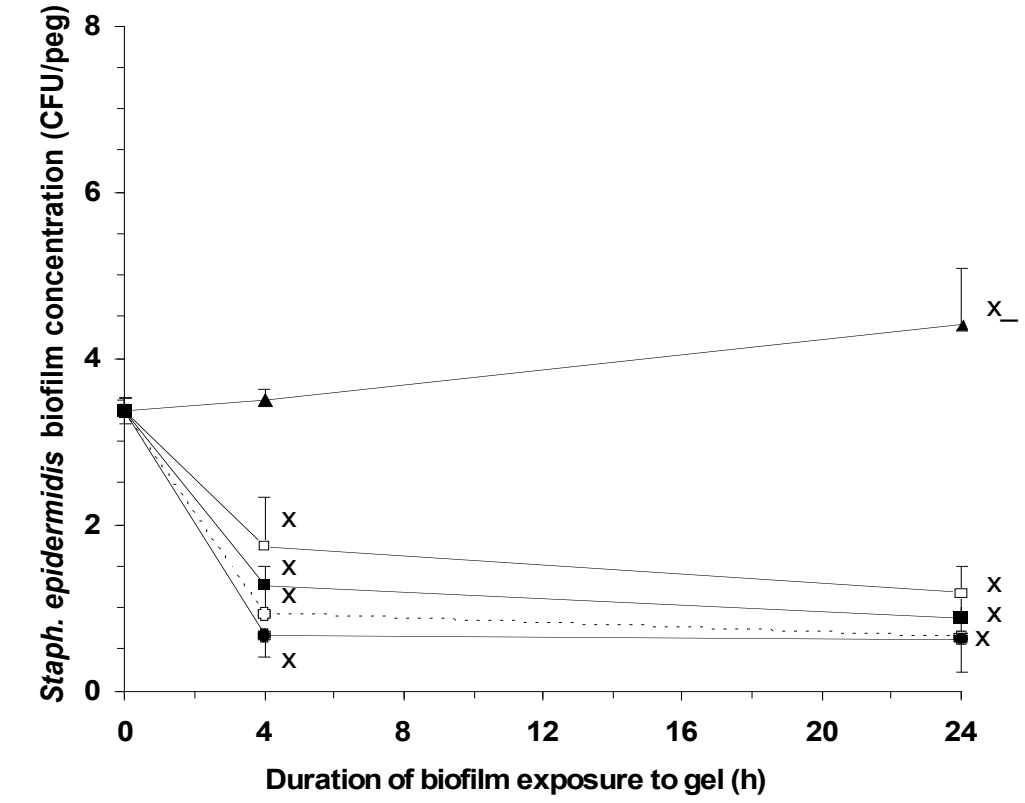

B.
Staph. epidermidis biofilm concentration (CFU/peg)
0
2
4
6
8
0
4
8
12
16
20
24
Duration of biofilm exposure to gel (h)
x_
x
x
x
x
x
x
x

Fig. 3

4. Discussion

Biofilm formation is an effective protective strategy adopted by bacteria to promote survival within hostile environments such as that encountered at the wound surface. It is well recognized that biofilm-associated infections are difficult to eradicate, as sessile bacteria employ mechanisms that raise survival and resistance to antimicrobial agents up to 1000 times compared with their planktonic counterparts [11, 23]. Our *in vitro* data clearly demonstrate the potential role for RescuDerm^{TM} as a biofilm-eradicating agent.

In vitro models measuring the minimum biofilm eradication concentration have been developed to provide practical tools for screening bactericidal and bacteriostatic agents against biofilms. However, their ability to accurately predict a drug's clinical efficacy is limited, as they do not encompass the complex conditions generated in host tissue *in vivo*. For example, Conley et al. [24] have recently shown that cephalosporins were less effective than penicillin against Group A *streptococcus* clinical isolates grown using the MBEC device, a finding in contrast to what is observed in the clinical setting [25]. Most *in vitro* susceptibility testing is performed on homogenous bacterial biofilms, but native biofilms often contain symbiotic mixtures of different species, and infections with organisms such as pasteurellosis or hemophilosis respond well to antimicrobial agents provided absence of a secondary pathogen [26]. The magnitude of *in vitro* bactericidal effects of antibiotics [25] and biocides [27] is also affected by the composition of growth media employed, the presence of serum proteins markedly reducing the antimicrobial action of these compounds. However, Cherrington et al. [28] have shown that the presence of blood and serum had no effect on the bactericidal

activity of organic acids. Similarly, we have previously shown that RescuDerm™ was very effective against bacteria despite the presence of 50% fetal calf serum in our germ carriers [16]. In the MBEC model system, the nutrient broths used to dilute the different gels contained several compounds that may nevertheless have affected the bactericidal efficacy of RescuDerm™. For example, Entani et al. [29] have reported that the combination of vinegar and sodium chloride had a highly synergistic effect on the low intrinsic bactericidal activity of vinegar, these compounds being ingredients of RescuDerm™ and the two general purpose media used, respectively. Studies are currently underway to assess the magnitude of RescuDerm's bactericidal properties in various models of contaminated full-thickness wounds.

We tested 24 hr old bacterial biofilms, as this growth period elicited maximal viable cell density, the latter parameter correlating with the formation of mature biofilms in several *in vitro* models, including the MBEC [17, 18, 30]. Harrison-Balestra et al. [31] have shown that a wound-isolated *Pseudomonas aeruginosa* grows a mature biofilm *in vitro* within 10 hr. While the maturation of a biofilm in the wounds may take longer, *in vitro* and *in vivo* biofilms share the same morphological characteristics. We observed a marked bacterial susceptibility after a 4-hr exposure of our biofilms to RescuDerm™. The subsequent increase in bacteria survival upon prolonging the biofilm exposure to most of the different amorphous gel formulations might be partly related to their failure to reach the bacteria within the biofilm community [10]. However, bacterial biofilms become harder to eradicate as they mature, and 24-hr sessile populations can be significantly less resistant to acetic acid than 7 days biofilms [32]. While our findings might suggest a role for RescuDerm™ for providing early infection control, further studies are required to

assess its role in treating chronic wounds and difficult-to-treat infections with mature biofilms.

Our finding that citric acid *plus* acetic acid were collectively effective in mediating the bactericidal effect of Rescuderm™ is in agreement with studies showing that topical application of such organic acids can successfully eradicate *Ps. aeruginosa* from burns and promote formation of granulation tissue [33, 34]. Their antibacterial efficacy has also been noted in treating infected root canals [35], venous leg ulcers [36], and various antibiotic-resistant superficial infections [37]. Our data suggest that citric acid and acetic acid were equally effective in exerting the bactericidal effect of Rescuderm™. However, citric acid exceeds the acetic acid/vinegar concentration in the formula (i.e., 4% vs. 1%), perhaps explaining that Abdul-Raouf et al. [38] found better *in vitro* bacteriostatic and bactericidal activity with acetic acid than citric acid alone. While small differences in pH were found between the acetic acid-free and citric acid-free Rescuderm™ gels, their equivalent potency might be related to the presence of comparable concentrations of undissociated acid molecules in solution [39]. As the bactericidal efficacy of RESC was reduced when using glacial acetic acid and given that the acidified placebo gel (pH 5) had little activity, it is conceivable that the observed inhibition of biofilm growth relates to the amount of undissociated acid molecules rather than merely pH. The antimicrobial effect of the sodium acetate salt-supplemented gel would be consistent with this possibility as well as published literature [40].

Our data suggest differential effects of EDTA and Carbopol® 940 on *Ps. aeruginosa* and *Staph. epidermidis* biofilm survival. However, our finding of an enhancement of the bactericidal effect of RescuDerm™ against *Ps. aeruginosa* when

removing both ingredients from the original formulation is complicated by the results of our preliminary studies that demonstrated adverse effects of raised viscosity in the MBEC system *per se*. Nagai et al. [41] reported that disodium EDTA was bactericidal against *Helicobacter pylori* but only during logarithmic growth. EDTA is a commonly used preservative due to its synergistic or potentiating action with other preservatives, antibiotics and cationic surfactants [42, 43]. Chiori et al. [44] reported that addition of 0.1% EDTA to nutrient broth was ineffective in killing either *Ps. aeruginosa* or *Staph. aureus*, this concentration being comparable to that in RescuDerm^{TM}. While we observed statistical significance, the small differences in biofilm survival of several modified RescuDerm^{TM} formulations might not be of clinical significance, as the viable bioburdens observed have not typically been associated with a greater risk of infection and failure of skin grafts [45]. Nevertheless, clinical trials assessing the usefulness of RescuDerm^{TM} in controlling the growth of mixed bacterial populations and/or that of more virulent microorganisms than those tested in the present study (e.g., beta hemolytic streptococci) are recommended.

In summary, these data expand our previous findings that RescuDerm^{TM} is an effective bactericidal agent, demonstrating that it controls the growth of common pathogenic biofilms. Its bactericidal efficacy appears to reflect the presence of weak, only partially dissociated organic acid residues in the gel formulation rather than merely formula pH. Taken together with the observation that RescuDerm^{TM} possesses broad *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, ON Canada; personal communication), this study

suggests the potential usefulness of this product for controlling biofilm formation on a variety of cutaneous traumatic and surgical wounds.

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Figure legends

Figure 1. Effect of alterations in pH and/or viscosity of the original RescuDerm™ formulation (RESC) on the formation of *Pseudomonas aeruginosa* (Panel A) and *Staphylococcus epidermidis* (Panel B) biofilms. Biofilms were exposed for either 4 hr or 24 hr to the diluted control broth (Negative control), placebo gels or modified RescuDerm™ gel formulations. Data are expressed as means ± SEM (n=6). Negative control values were different from all other treatments throughout the study. * Different from original RESC ($p<0.05$) † Different from placebo pH 5 ($p<0.05$) ‡ Different from placebo pH 7 ($p<0.05$) § Different from RESC pH 7 normal viscosity Different from previous time interval ($p<0.05$) x Different from initial biofilm value ($p<0.05$)

Figure 2. *Pseudomonas aeruginosa* (Panel A) and *Staphylococcus epidermidis* (Panel B) biofilm formation following either a 4 hr or 24 hr exposure to citric acid- and/or acetic acid-free RescuDerm™. Data are expressed as means ± SEM (n=6). Negative control values were different from all other treatments throughout the study. $C_2H_3NaO_2$ Sodium acetate $C_2H_4O_2$ Glacial acetic acid * Different from original RESC ($p<0.05$) † Different from citric acid-free RESC ($p<0.05$) ‡ Different from acetic acid-free RESC ($p<0.05$) § Different from acetic acid-free/C2H3NaO2-supplemented RESC Different from previous time interval ($p<0.05$) x Different from initial biofilm value ($p<0.05$)

Figure 3. *Pseudomonas aeruginosa* (Panel A) and *Staphylococcus epidermidis* (Panel B) biofilm survival following either a 4 hr or 24 hr exposure to Carbopol®- and/or EDTA-free RescuDerm™. Data are expressed as means ± SEM (n=6). * Different from

original RESC ($p<0.05$) □ Different from previous time interval ($p<0.05$) ˣ Different from initial biofilm value ($p<0.05$)

Table 1. Physico-chemical characteristics of the different gel formulations tested using the MBEC™ device

Code	Gel description	Characteristics			
		Viscosity	*pH*		
			Undiluted	**PSEUD 1:4**	**STAPH 1:8**
A	Citric acid-free	27,000	$4.00 \pm 0.02^{e-i}$	$4.55 \pm 0.04^{c,f,i}$	$5.02 \pm 0.04^{\dagger\ c-f}$
B	Acetic acid-free	9,200	$4.03 \pm 0.05^{e,f,h,i}$	$4.50 \pm 0.03^{c,\ e-i}$	$4.90 \pm 0.05^{\dagger\ e-g,\ i}$
C	Carbopol®-free	0	$3.94 \pm 0.04^{e-i}$	$4.40 \pm 0.02^{d-i}$	$4.77 \pm 0.01^{\dagger\ e-i}$
D	Acetic acid-free + $C_2H_3NaO_2$	10,700	$4.04 \pm 0.04^{e-i}$	$4.48 \pm 0.01^{e,\ h,\ i}$	$4.89 \pm 0.03^{\dagger\ e-i}$
E	Neutral pH RescuDerm™	30,400	$6.84 \pm 0.08^{g-i}$	$7.21 \pm 0.05^{f-i}$	$7.39 \pm 0.04^{\dagger\ f-i}$
F	RescuDerm™ with same viscosity as original RESC	8,000	$7.05 \pm 0.06^{g-i}$	$7.30 \pm 0.04^{g-i}$	$7.45 \pm 0.03^{\dagger\ g-i}$
G	Acetic acid- & citric acid-free + $C_2H_4O_2$	96,000	$4.15 \pm 0.03^{h-i}$	4.71 ± 0.03	$5.17 \pm 0.08^{\dagger}$
H	EDTA- & carbopol®-free	0	4.30 ± 0.00	4.64 ± 0.0^{i}	$5.02 \pm 0.01^{\dagger\ i}$
I	EDTA-free	980-1240*	$4.36 \pm 0.01^{*}$	4.70 ± 0.02	$5.08 \pm 0.02^{\dagger}$
R	Original RescuDerm™	7000-13000*	$4.21 \pm 0.04^{a-f,\ i}$	$4.63 \pm 0.04^{b-f}$	$4.95 \pm 0.10^{\dagger\ b,\ e,\ f}$
P5	Placebo	N/A	$5.01 \pm 0.04^{a-d,\ g-h,\ R}$	$5.01 \pm 0.04^{R,a-i}$	$5.02 \pm 0.04^{\dagger\ d-f}$
P7	Placebo	N/A	$7.32 \pm 0.14^{\ a-I,\ R,\ P5}$	$7.28 \pm 0.09^{R,\ a-d,\ g-i,\ P5}$	$7.56 \pm 0.07^{\dagger\ R,\ a-e,\ g-i,\ P5}$

^{C2H3NaO2} Sodium acetate ^{C2H4O2} Glacial acetic acid ^{EDTA} Ethyldiaminetetraacetic acid ^{MBEC} Minimum Biofilm Elimination Concentration. $^{N/A}$ Not provided by the manufacturer. * Product specifications. Data are expressed as means ± SEM (n=6). * Different from diluted gels ($p<0.05$) ^{Letter} Different from a given gel within a given dilution ($p<0.05$) † Different from other diluted gel ($p<0.05$)