Antibiofilm Properties of Acetic Acid

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Bacterial biofilms are known to be extremely tolerant toward antibiotics and other antimicrobial agents. These biofilms cause the persistence of chronic infections. Since antibiotics rarely resolve these infections, the only effective treatment of chronic infections is surgical removal of the infected implant, tissue, or organ and thereby the biofilm. Acetic acid is known for its antimicrobial effect on bacteria in general, but has never been thoroughly tested for its efficacy against bacterial biofilms. In this article, we describe complete eradication of both Gram-positive and Gram-negative biofilms using acetic acid both as a liquid and as a dry salt. In addition, we present our clinical experience of acetic acid treatment of chronic wounds. In conclusion, we here present the first comprehensive *in vitro* and *in vivo* testing of acetic acid against bacterial biofilms.

INTRODUCTION

Microbes, in particular bacteria, are known to cause various types of infections in both humans and animals. Antibiotics are usually the choice of treatment for infections and can be used to either kill or inhibit the growth of the microbes. However, the worldwide increase in antibiotic-resistant microbes has limited the effect of traditional treatments making it very difficult to treat infections that were once treatable. A particular problem is infections where the bacteria are capable of forming a biofilm, which is an aggregate of bacteria.[1] Here, the bacteria are tolerant to antibiotics and most other antimicrobial agents.[2] Microbial biofilm infections may be discerned from acute bacterial infections by (1) persistence despite antibiotic therapy and the innate and adaptive immune and inflammatory responses of the host and (2) in contrast to colonization, are characterized by a proinflammatory immune response and persisting pathology.[3]

Biofilms are known to be involved in many chronic infections such as in chronic wound infections, chronic lung infections in cystic fibrosis patients, chronic otitis media, endocarditis, rhinosinusitis, chronic obstructive pulmonary disease, and catheter- or implant-related infections.[4] A more commonly known phenomenon with biofilm pathology is oral plaque[5–7] and it is also a problem in the food industry production lines.[8,9]

CLINICAL PROBLEM ADDRESSED

Due to the increase in resistant bacterial strains and the inherent

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tolerance of biofilm infections, it is important to devise new treatment scenarios, which efficiently enable eradication of infecting microorganisms.[2]

Acetic acid is present in vinegar in a 3–5% concentration, which has been used in medicine for thousands of years. Hippocrates recommended it for food preservation and as a tonic, as well as to treat wounds.[10] It is a weak organic acid and can be inexpensively produced either by fermentation from ethanol or synthetically. As a drug it is favorable since it can easily be autoclaved and stored. The US Food and Drug Administration has approved acetic acid in a 0.25% solution for bladder irrigation and in a 2% solution for treating external otitis. The usage of acetic acid for wound treatment was described almost 100 years ago for treatment of war-wound infections. In 1916, Taylor[11] successfully used a 1% acetic acid solution against *Bacillus pyocyaneus*, now known as *Pseudomonas aeruginosa*, in war-wound infections in 111 patients. Since then, several articles have been published describing acetic acid for topical treatment.[12–22] In addition, acetic acid has been used with success for different types of otitis media.[23]

We have discovered that not only does acetic acid kill planktonic bacteria but it also eradicates bacteria growing in biofilms. Acetic acid is a liquid at ambient pressure and temperature, and therefore any compositions comprising acetic acid are liquid or wet compositions. However, the salt sodium diacetate ($NaHAc_2$) leads to acetic acid when dissolved in liquid and is equally effective at killing biofilm-forming bacteria.

In this article, we describe the usage of acetic acid alone and in combination with other antibacterial agents, such as tobramycin, ciprofloxacin, and colistin. In addition, we also describe the usage of acetic acid in combination with negative pressure wound therapy (NPWT).

MATERIALS AND METHODS

The wild-type *P. aeruginosa* PAO1 used for the planktonic and biofilm experiments was obtained from the Pseudomonas Genetic Stock Center (www.pseudomonas.med.ecu.edu, strain PAO0001). The wild-type *Staphylococcus aureus* 8325-4, used for planktonic and biofilm experiments, was described by Novick.[24] The following bacteria were obtained from the Department of Clinical Microbiology, Copenhagen University Hospital, Denmark: *Escherichia coli* (wild-type and clinical Extended-Spectrum Beta-Lactamases (ESBL)-producing strains), *S. aureus* (wild-type and clinical Methicillin-resistant *Staphylococcus aureus* (MRSA) strains), and *Klebsiella pneumoniae* (clinical ESBL-producing strain).

Growth media

For plating, the Luria Broth (LB) medium mix with 2.0% agar (Substrate Department at the Panum Institute, Copenhagen, Denmark) was used. For all experiments, including bacterial biofilms, the AB minimal medium supplemented with glucose was used except if difference is mentioned. The AB minimal medium consists of a standard buffer system comprising $(NH_4)_2SO_4$ (15.1 mM), $Na_2HPO_4 \cdot 2H_2O$ (33.7 mM), KH_2PO_4 (22.0 mM), NaCl (0.051 M), $MgCl_2$ (1 mM), $CaCl_2$ (0.1 mM), and trace metals (100 μL/L). The trace metal solution contained $CaSO_4 \cdot 2H_2O$ (200 mg/L), $FeSO_4 \cdot 7H_2O$ (200 mg/L), $MnSO_4 \cdot H_2O$ (20 mg/L), $CuSO_4 \cdot 5H_2O$ (20 mg/L), $ZnSO_4 \cdot 7H_2O$ (20 mg/L), $CoSO_4 \cdot 7H_2O$ (10 mg/L), $NaMoO_4 \cdot H_2O$, and H_3BO_3 (5 mg/L).

Growth of bacteria

Three types of biofilm setups were used, a continuous flow system, a static microtiter plate system, and a static filter biofilm assay:

The continuous flow system is based on once-through flow chambers perfused with the sterile AB minimal medium containing 0.3 mM glucose, as described by Christensen *et al.*[25]

The microtiter plate assay is based on biofilms growing in microtiter dishes with the AB minimal medium containing 0.3 mM glucose, as described by O'Toole and Kolter.[26]

The micropore assay is based on biofilms growing on a micropore filter on AB minimal agar plates. The micropore filters are placed on top of an AB minimal agar plate. Bacteria are propagated on the micropore filters as spots of 20 μL bacterial suspensions and incubated at 37°C. For a mature biofilm to develop, the filters were transferred to a fresh AB minimal agar plate every 24 h. Treatments were applied to the biofilms after either a 20-h (immature biofilm) or 168-h (mature biofilm) incubation in total, at 37°C.

Planktonic cultures were grown in shake flasks at 37°C. The microtiter plate assay for biofilm tolerance to $NaHAc_2$ was assessed by exchanging the AB minimal medium of 24-h old biofilms with the AB minimal medium supplemented with different concentrations of $NaHAc_2$.

Antimicrobial treatments

Continuous flow biofilm tolerance to acetic acid was assessed by growing *P. aeruginosa* or *S. aureus* biofilms for 3 days, then subsequently at day 3–4 supplementing the AB minimal medium with different concentrations of acetic acid or HCl (Sigma-Aldrich) as a control to acetic acid treatments. Due to the buffer capacity of the AB minimal medium used, the pH of the solution became 4.33. As

control, similar biofilms were treated with the AB minimal medium adjusted to pH 4.33 using HCl.

Static biofilm tolerance to acetic acid was assessed by exchanging the AB minimal medium of 24-h old biofilms with the AB minimal medium, supplemented with different concentrations of acetic acid or HCl as control. To raise the pH of either acetic acid or HCl, NaOH (Sigma-Aldrich) was added in different concentrations. For the combinatory treatment of acetic acid and antibiotics, the solutions of acetic acid and tobramycin were adjusted to the desired pH, as described above.

In the micropore filter biofilm assay, $NaHAc_2$ was added on top of the biofilm spot (after 48 h), either directly or between two pieces of gauze. The control was plain gauze. All treatments were incubated for 24 h. The effect was evaluated by replication of the micropore filter after treatment. The endpoint was growth or no growth of bacteria after the treatment when replica plated onto a fresh LB plate.

To verify whether the biofilms grown in the used model systems were tolerant to antibiotics, suspensions of tobramycin (100 μg/mL), ciprofloxacin (100 μg/mL), and colistin (25 μg/mL) (final concentrations) (Region Hovedstadens Apotek) were individually added in the different biofilm models. Neither of the antibiotics had a significant effect on the killing of the bacteria.

All studies of antimicrobial effect were performed in triplicates or more.

NPWT and acetic acid

The combination of NPWT and acetic acid has been used in our clinic for more than 5 years and more than 200 patients have been treated. The indications for use have had a broad range and have been used as a treatment modality in the clinic where further surgical debridement was not likely to eradicate the infection or would deteriorate function. The patients were treated off-label with acetic acid. The selection of patients was done on clinical presentation. Especially, the recurrence of soft tissue deterioration despite antibiotic treatment and/or surgical debridement would be an indication for treatment with acetic acid. Another indication has been chronic *P. aeruginosa* soft tissue infection. All patients in this case study series had a local chronic infection with or without foreign material in the area. If a deeper infection is suspected, we additionally treat with systemic antibiotics. So far, the conditions treated have been diabetic foot ulcers, venous leg ulcers, postoperative infections with exposed osteosynthesis, and articular infections. The device used to deliver the acetic acid and to provide the NPWT is the VAC Veraflo® (Kinetics Concept International). This device has the dual modality of providing NPWT and soaking of appropriate fluid on an alternate basis. The device can be programmed with different settings with respect to time of NPWT, time of soaking of fluid, and volume of delivered fluid. In addition, it can deliver different values of negative pressure.

Method

All patients have an initial, thorough surgical revision where representative biopsies for culture are obtained. Then, the NPWT dressing is applied either at once or the following day. Bleeding sometimes prevents the dressing to be applied postoperatively. Standard settings of the device are 3.5 h of NPWT, 20 min of soak, and a negative pressure of 125 mmHg. This cycle is repeated and results in six cycles per day. The volume of the solution is adjusted according to the volume of the wound and the dressing. The dressing is changed every fourth to fifth day and the wound bed is inspected. The usual length of treatment is 9 days with a range from 5 to 21. The applied solutions are a 1% acetic acid solution in isotonic saline until November 2013, and hereafter, a 1% normo-osmolar acetic acid solution with a PBS buffer adjusted to pH 4.7 as this solution became available from our pharmacy.

Statistics

All statistical analyses were performed with GraphPad Prism version 6.00 for Macintosh, GraphPad Software (www.graphpad.com). Data were considered significant with $p < 0.05$. Significance levels are depicted as ****Extremely significant, < 0.0001; ***Extremely significant, 0.0001–0.001; **Very significant, 0.001–0.01; *Significant, 0.01–0.05; Not significant (ns), ≥ 0.05.

RESULTS

The effect of acetic acid is effective against mature biofilms of P. aeruginosa *and* S. aureus

Treating a 3-day-old flow chamber biofilm of *P. aeruginosa* or *S. aureus* with 0.5%, 1.0% acetic acid or HCl as control for 24 h tested the efficacy of acetic acid with respect to eradication of mature biofilms. After the treatment, the biofilm biomasses were harvested mechanically from the flow chambers and plated on LB plates for determination of viability. Treatment with either 0.5% or 1.0% acetic acid completely eradicated *P. aeruginosa* biofilms (*i.e.,* no viable counts on plates), whereas the HCl treatment had no effect. As for *S. aureus*, treatment with 0.5% acetic acid reduced the number of viable cells, whereas complete eradication was obtained using 1.0% acetic acid (Table 1).

Table 1. *Semiquantitative evaluation of harvested biofilm from flow cells after treatment*

Treatment	*0.5% acetic acid*	*1% acetic acid*	*HCl*
Pseudomonas aeruginosa	100% eradication	100% eradication	Growth
Staphylococcus aureus	Partial eradication	100% eradication	Growth

Three-day-old flow cell biofilms of *P. aeruginosa* or *S. aureus* were treated with 0.5%, 1.0% acetic acid or HCl as control for 24 h to test the efficacy of acetic acid with respect to eradication of mature biofilms.

To verify whether the killing capacity of the treatment was due to acetic acid alone and not a combination of the constituents of the medium, the above experiment was repeated using 0.5% or 1.0% acetic acid in sterile miliQ water in contrast to the AB minimal medium supplemented with glucose. Complete eradication was observed when harvesting 0.5% acetic acid-treated *P. aeruginosa* and 1.0% acetic acid-treated *S. aureus* biofilms compared with the controls.

To evaluate the clinical antimicrobial potential of acetic acid, static biofilms of clinical *P. aeruginosa* isolates were treated with acetic acid. Clinical isolates of both mucoid and nonmucoid *P. aeruginosa* strains were completely eradicated by acetic acid below pH 4.35 (results not shown).

The effect of pH on the antimicrobial properties of acetic acid

To elucidate the pH dependency of the antimicrobial effect of acetic acid, static 24-h-old cultures were treated with a selection of 0.5% acetic acid solutions with increasing pH. Acetic acid in the AB minimal medium resulted in a pH value of 4.33, higher pH values were achieved by addition of NaOH. This was compared with AB minimal media adjusted to the same range of pH by HCl or NaOH alone. We found a complete killing of all bacteria in the wells when the pH was lower or equal to 4.33. A Kruskal–Wallis test revealed that pH had a significant influence on the antimicrobial effect of acetic acid. Dunn's posttest was applied to find significant differences to the control (minimal media) at specific pH values. As seen in Fig. 1A, acetic acid had a significant antimicrobial effect against *P. aeruginosa* at pH below 4.76. Above pH 5, only minor nonsignificant effects were observed.

This pH dependency is due to the dissociation of acetic acid, which is at equilibrium at pH 4.76 (pKs value). Below pH 4.76, the equilibrium is shifted to the left, that is, acetic acid; above pH 4.76, the equilibrium is shifted to the right, that is, the corresponding base.

$$CH_3COOH \rightarrow CH_3COO^- + H^+ (pH > 4.76)$$
$$CH_3COOH \leftarrow CH_3COO^- + H^+ (pH < 4.76)$$
$$CH_3COOH = CH_3COO^- + H^+ (pH = 4.76)$$

HCl does not exert a similar effect on microbial growth as acetic acid even when the two compounds are used at the same pH (Fig. 1B). The results of the present experimental scenario demonstrate that it is not an acidic effect (*i.e.,* low pH values) *per se* that causes the kill; it is the acetic acid molecule itself in its nondissociated form.

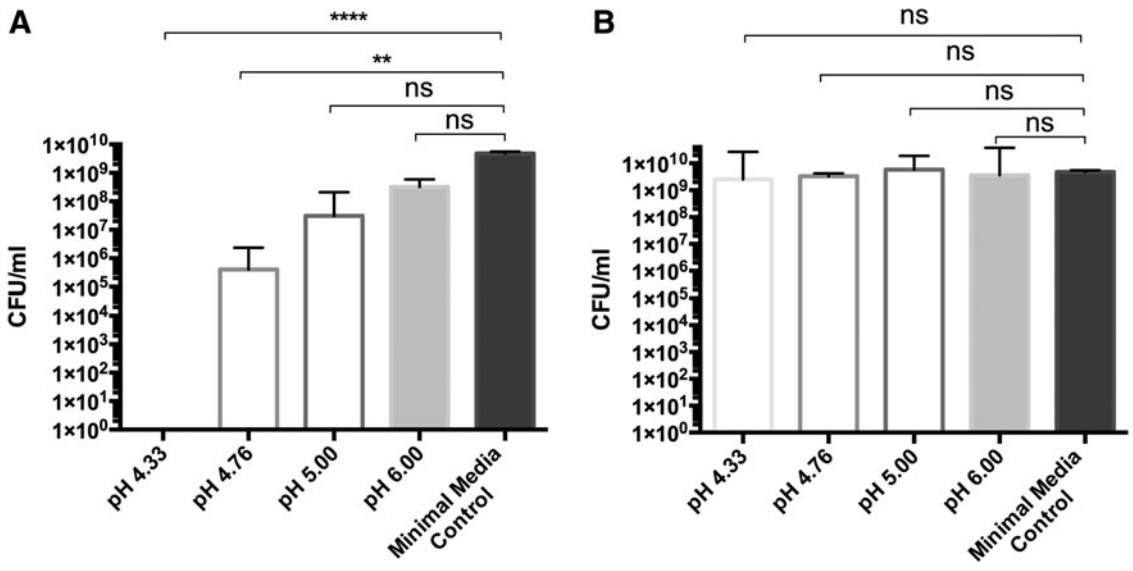

Figure 1. **(A)** Viable counts of a 24-h-old static culture of *Pseudomonas aeruginosa* treated with acetic acid for 24 h at a range of pH. **(B)** Viable counts of a 24-h-old static culture of *P. aeruginosa* treated with hydrochloric acid for 24 h at a range of pH. A Dunn's post-test was performed to find significant difference between the treated samples and the control. *Bars* represent median with interquartile range. $N = 6$. *Indicates significance and ns indicates nonsignificant differences (see section on statistics).

In addition to acetic acid, we also tested the antibacterial ability of other weak organic acids, including citric acid, methane acid, propane acid, butane acid, and boric acid, with similar results (data not shown). However, these acids are not of medical interest due to severe odor disadvantages.

Combinatory treatment of acetic acid and antibiotics

To elucidate the antimicrobial effect of combining acetic acid and antibiotics, static biofilms of *P. aeruginosa* were grown for 24 h before subsequent treatment with increasing concentrations of tobramycin and 0.5% acetic acid in the pH interval 4.33–6.00 (Fig. 2). The pH was adjusted by supplementing the AB minimal medium with NaOH.

A two-way ANOVA found that the contribution of tobramycin, pH, and their interaction was significant. The pH was found to account for 51% of the variance between the groups ($p < 0.0001$) and tobramycin was found to account for 7.31% ($p = 0.0002$). In the control experiment with HCl, only tobramycin contributed significantly to the variance (12.41% contribution; $p = 0.0045$). Interestingly, the test suggested a significant interaction between pH and tobramycin in the presence of acetic acid (14.64% contribution; $p < 0.0001$), which was not found in the presence of HCl. Thus, it seems that the combination of acetic acid and tobramycin elicits an enhanced effect.

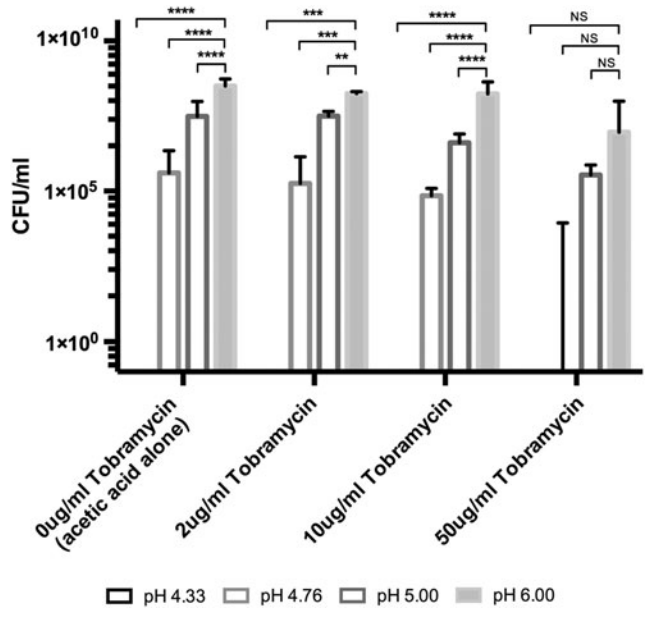

Figure 2. Viable counts of a 24-h-old static culture of *Pseudomonas aeruginosa* treated with acetic acid and tobramycin for 24 h at a range of pH. Tukey's multiple comparisons test followed the two-way ANOVA to determine significant differences between each pH value (Fig. 2). *indicates significance and ns indicates nonsignificant differences (see section on statistics). *Bars* represent median with interquartile range. $n = 6$.

Tukey's multiple comparisons test followed the two-way ANOVA to determine significant differences between each pH value (Fig. 2).

In addition to tobramycin, the antibacterial substances ciprofloxacin and colistin were also tested and displayed analogous synergistic effects (results not shown).

Sodium diacetate

pH dependence of sodium acetate concentration. Since acetic acid itself is a liquid and potentially difficult to use in a wound dressing, we identified $NaHAc_2$ as a dry source of acetic acid when dissolved in liquid. To measure the correlation between dissolved $NaHAc_2$ and pH, increasing amounts (w/v) were added to AB minimal media. The measurements show a decrease in pH with increasing amounts of $NaHAc_2$, as depicted in Table 2.

Concentration dependence of $NaHAc_2$ kill rate. To elucidate the concentration dependency of the antimicrobial effect of $NaHAc_2$, static 24-h-old biofilms grown in microtiter plates were treated for another 24 h with increasing amounts of $NaHAc_2$, as depicted in Table 2, with the decrease in pH, as seen in Table 2. The antimicrobial effect was determined by plating the treated and nontreated biofilms. To ensure complete removal of all the adhering bacteria, the wells of the microtiter plate were washed mechanically. We observed a complete killing effect of the biofilm using 1.75% (w/v) added $NaHAc_2$ and higher. Surprisingly, we did not observe the strict pH-dependent killing for $NaHAc_2$, which we had previously observed using acetic acid.

Effect of dry $NaHAc_2$ on microbial biofilm. To elucidate the efficacy of dry $NaHAc_2$on microbial

Table 2. *The decrease in pH and average CFU with increasing amounts of NaHAc2, on micropore filter biofilms*

% $NaHAc_2$ (w/v)	*Media pH*	*Median CFU*	*SD*
0.00%	6.82	1.30E + 10	1.23E + 10
0.50%	5.78	2.68E + 08	3.20E + 08
0.75%	5.31	2.33E + 08	1.53E + 08
1.00%	5.09	2.13E + 07	7.78E + 06
1.25%	4.99	2.18E + 05	3.05E + 05
1.50%	4.91	1.70E + 03	2.46E + 02
1.75%	4.86	0	
2.00%	4.83	0	
2.25%	4.80	0	
2.50%	4.78	0	
2.75%	4.76	0	
3.00%	4.74	0	

$NaHAc_2$, sodium diacetate.

biofilms of *P. aeruginosa,* we propagated the bacteria on a micropore filter for biofilm formation (Fig. 3). We tested the effect on both immature biofilms (20-h old) and very mature biofilm (168-h old) to validate the effect on both growing biofilms surrounded by a little matrix and nongrowing biofilms surrounded by a massive matrix.

As seen in Fig. 3, the presence of $NaHAc_2$ completely eradicates biofilms of both ages and can thus eradicate biofilms with slow growth protected by a thick matrix, which usually impedes most antimicrobial compounds. This is in contrast to the control, which was not affected by the gauze.

The *in vitro* effect of dry $NaHAc_2$ on *P. aeruginosa* was confirmed for biofilms of *E. coli* (wild-type and clinical ESBL-producing strains), *S. aureus* (wild-type and clinical MRSA strains), and *K. pneumoniae* (clinical ESBL-producing strain) (data not shown).

Clinical use of acetic acid

To evaluate the clinical potential of 1% acetic acid solution toward biofilms in chronic infections, it has been used in our clinic as a treatment modality, as described previously in this article. The indication for use of acetic acid has been in cases where standard wound care, repeated debridement, and even amputation have not lead to viable and healthy granulation. Other cases have exposed foreign material as osteosynthesis, osteomyelitis, or joint infections. Not all cases have been successful. In cases where only a part of the foreign

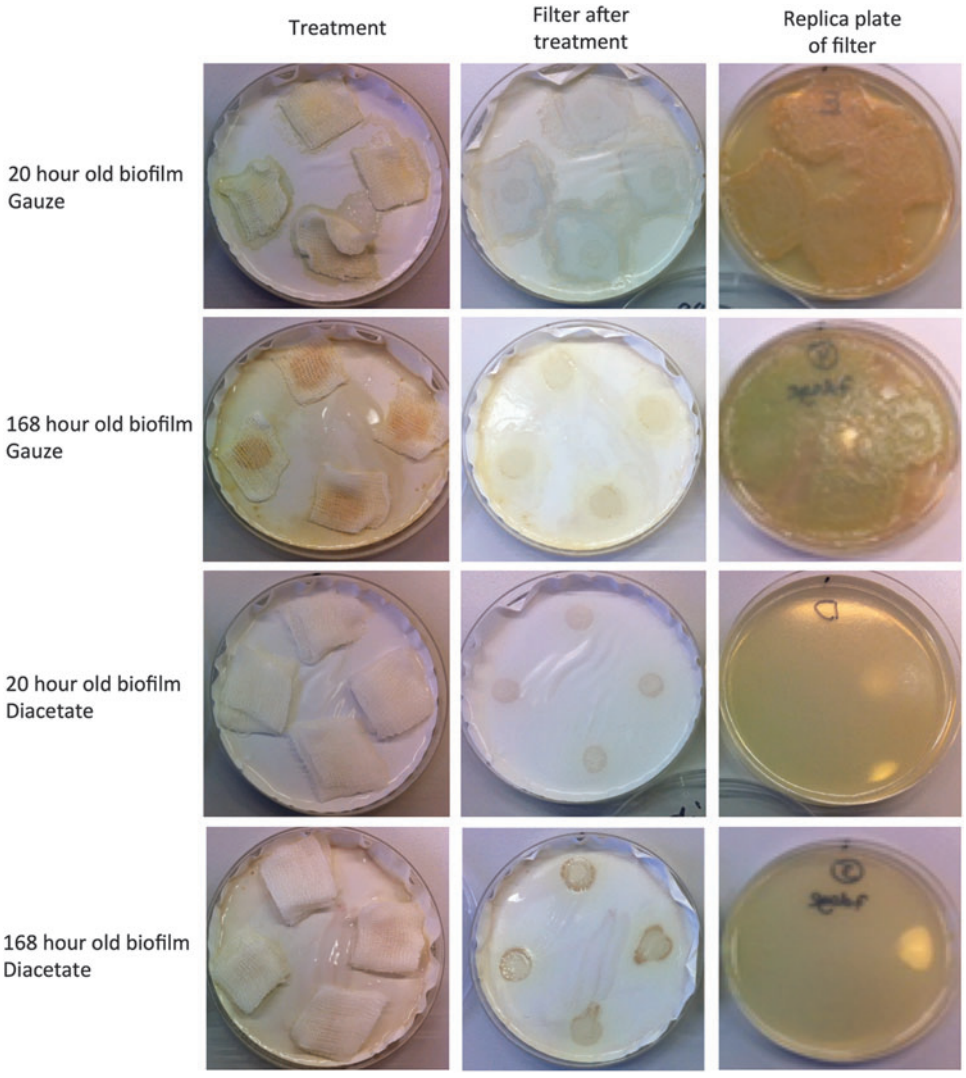

Figure 3. The effect of adding sodium diacetate between a gauze soaked in wound buffer to mature biofilms grown on a micropore filter. Several layers of gauze soaked in wound buffer served as control. Biofilms were grown on Millipore filters on AB minimal agar plates for either 20 or 168 h to represent both immature and mature biofilms. Several layers of gauze were soaked in wound buffer before application of dry sodium acetate. Fresh gauze with diacetate or wound buffer alone (control) was applied after 24 h and, thus, the total treatment lasted 48 h before evaluation by replica plating. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

material had been exposed, the local treatment with acetic acid could not eradicate the infection in the remote nonexposed area. In this study, we provide three examples.

Example 1

Medical history. A 38-year-old male with type 2 diabetes mellitus-associated neuropathy presented to a wound-healing clinic. A heel ulcer was obtained during a vacation due to strenuous walking. The patient had the following history of treatment with no apparent improvement in wound healing (over a period of 3 months): off-loading, therapeutic shoes, and air cast; wound treatment with silver dressings and compression; and several courses of antibiotics.

Treatment. Treatment of the chronic wound (Fig. 4A [day 0]) with 1% acetic acid (patient continued antibiotic therapy) was performed 6×20 min per day in combination with NPWT for 11 days (continuous). On day 11, the infection had been eradicated and wound healing had begun, as shown in Fig. 4B (day 11).

Example 2

To evaluate the clinical potential of acetic acid solution toward the clinically important, mucoid (alginate overproducing) *P. aeruginosa* phenotype, it was tested in the treatment of a chronic leg ulcer.

Medical history. A 73-year-old female with type 2 diabetes mellitus and a 3-year-long history of leg ulcers. The patient had received an ulcer debridement and split-skin transplant in 2006 with only temporarily success as the ulcer reoccurred after 3 months. The patient was considered unfit for a new operation due to her heart condition. Cultures before treatment have shown *P. aeruginosa* and *S. aureus*. The patient received anti-*Staphylococcus* treatment due to an infected toe on the contralateral leg (flucloxacillin).

Treatment. Treatment of wound (Fig. 5A [day 0]) with acetic acid 1% (patient continued antibiotic therapy) was performed 6×20 min per day, for 6 days (continuous) in combination with NPWT. After 3 days, a significant improvement in the wound healing process was evident, as shown in Fig. 5B (day 3), and after 6 days, the infection was eradicated and the wound healing process was proceeding well, as shown in Fig. 5B (day 6), and reoccurrence was not observed.

Example 3

To further evaluate the clinical potential of acetic acid solution toward the clinically important mucoid (alginate overproducing) *P. aeruginosa* phenotype, it was tested in the treatment of a chronic foot ulcer.

Medical history. A 53-year-old female, past drug addict, with a year-long history of lower leg ulceration. The patient had been treated with compression bandages for years and numerous antibiotic courses. The presented ulcer was very painful.

Treatment. The treatment was performed essentially as in example 3, that is, 6 treatments per day with acetic acid followed by application of negative pressure between those treatments. Figure 6 shows the clinical presentation before acetic acid treatment (Fig. 6A), the device used for NPWT application (Fig. 6B) and after 6 days of treatment (Fig. 6C). The ulcer improved dramatically with compression therapy even after cessation of active therapy.

DISCUSSION

Most, if not all, antibiotics and antiseptics fail to eradicate mature biofilms, and today, the poor

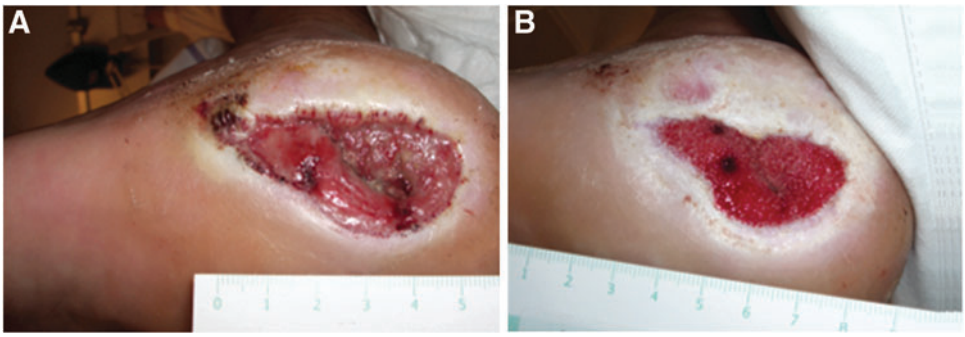

Figure 4. **(A)** A chronic heel ulcer infected with *Pseudomonas aeruginosa* in a 38-year-old male patient with type 2 diabetes mellitus before treatment with negative pressure wound therapy (NPWT) and acetic acid solution (day 0). At this stage, several well-known antibacterial treatments had been tried. **(B)** The heel ulcer after 11 days of treatment with NPWT and acetic acid solution (day 11). The mucoid infection has been eradicated and the ulcer is now healing normally. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

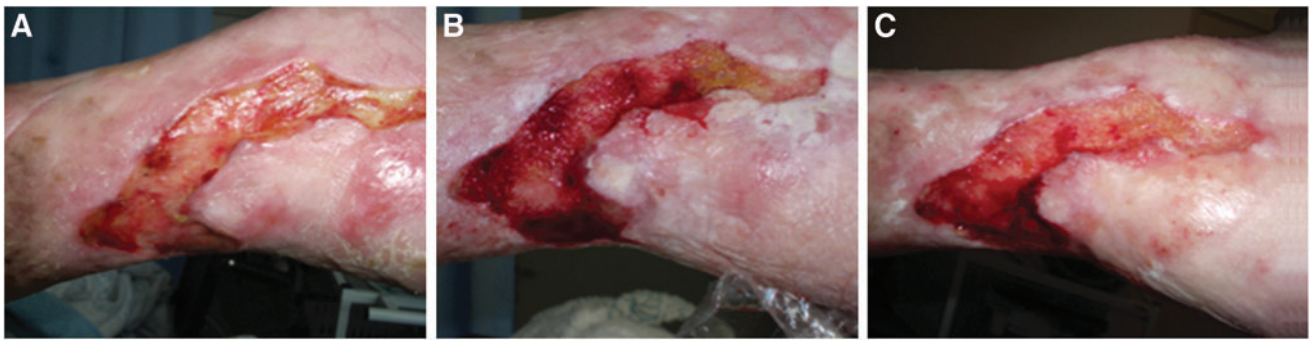

Figure 5. **(A)** A chronic leg ulcer infected with *Pseudomonas aeruginosa* in a 73-year-old female patient with type 2 diabetes mellitus before treatment with NPWT and acetic acid solution (day 0). Cultures before treatment have shown *P. aeruginosa* and *Staphylococcus aureus*. The patient received anti-*Staphylococcus* treatment due to an infected toe on the contralateral leg (Flucloxacillin). **(B)** The leg ulcer after 3 days of treatment with NPWT and acetic acid solution (day 3). The mucoid infection has been significantly reduced and the ulcer has attained a dark red color indicating reestablishment of the normal wound healing process. **(C)** The leg ulcer after 6 days of treatment with NPWT and acetic acid solution (day 6). The mucoid infection has been eradicated and the ulcer is now healing. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

efficiency of available antibiotics is a major challenge for the successful treatment of chronic infections.[2] At present, the most efficient treatment for biofilm infection is to mechanically remove the infected area or body part.[27] This is sometimes possible if the focus is a catheter, an implant, or an infected organ that is eligible for transplantation. However, the risk of complications for patients associated with surgical removal creates increasing demands for antibiotics with potential to eradicate biofilms. So far, the two main strategies for preventing or suppressing bacterial biofilm infections are (1) early aggressive antibiotic treatment before the biofilm is formed or (2) chronic suppressive antibiotic treatment when the biofilm is established, if it cannot be removed physically.[27]

In this study, we demonstrate that biofilms can be eradicated, by the simple use of acetic acid. We found that it is not the decrease of pH itself that kills the bacteria, as lowering of the pH with HCl to 4.76 and less did not result in antimicrobial activity. Our observation of maximal antibacterial effect at pH 4.76 and lower demonstrates that the antibacterial effect results from the nondissociated form of acetic acid (CH_3COOH) and not from the acetate (CH_3COO^-) derived by proton dissociation. Equal amounts of CH_3COO^- and CH_3COOH will exist at the pKs value at 4.76, and to maintain acetic acid in its bactericidal effective state, pH must be kept lower or close to 4.76. Thus, our observation of maximal antibacterial effect at pH 4.76 and lower demonstrates that the antibacterial effect results from acetic acid.

Acetic acid is considered harmless below concentrations of 5%, as in vinegar, but in concentrations between 10% and 30% acetic acid is corrosive. In addition, it has been observed that acetic acid among other tested antiseptics (5% mafenide acetate [Sulfamylon solution], 10% povidone with 1% free iodine [Betadine], 0.25% sodium hypochlorite [half-strength Dakin], 3% hydrogen peroxide, and 0.25% acetic acid) did not influence *in vivo* re-epithelialization.[28] In our clinical usage, we used 1% acetic acid with a very positive effect and al-

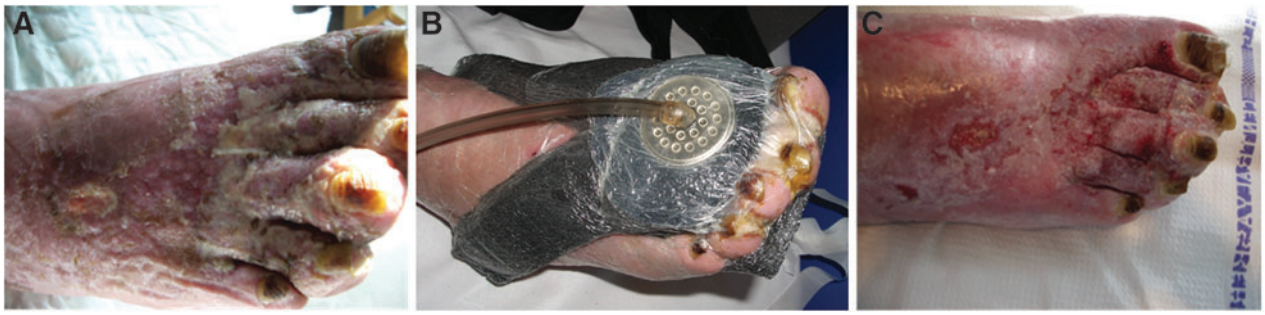

Figure 6. **(A)** A foot ulcer infected with *Pseudomonas aeruginosa* of a 53-year-old female, with a year-long history of lower leg ulceration, before treatment. **(B)** The wound dressing applied on the foot ulcer. **(C)** The foot ulcer after 6 days of treatment with NPWT and acetic acid solution (day 6). The mucoid infection has been eradicated and the ulcer is now healing. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

though it has not been in a controlled setting, we can conclude that the acetic acid treatment did not have a toxic effect on the tissue and it seems to be helpful in the treatment of hard-to-heal wounds. All the three cases presented have a treatment record that included several antibiotic treatments that were unsuccessful, but with the adjuvant treatment of a local biofilm-active compound like acetic acid, the treatment seemed much more effective. The NPWT has some effect on the biofilm itself,[29] but the clinical cases presented here would not be considered suitable for NPWT without instillation. NPWT with instillation has the potential to deliver several different solutions to the wounds. The present article cannot claim that the treatment with acetic acid together with NPWT is superior to any other treatment, but the results are very encouraging. The treatment has been used in several clinical situations that range from soft tissue infections over infected osteosynthesis to even in joint infections; however, as promising as the results appear, we need a controlled trial to conclude that the acetic acid is better than, for example, saline. For wound dressings, the incorporation of the salt $NaHAc_2$ would be perfect. This would enable a dry ready-to-use dressing. $NaHAc_2$ will be dissolved and acetic acid released when the dressing comes in contact with the moist wound enabling eradication of the biofilm-growing bacteria in the wound, as seen in Fig. 3.

In conclusion, we here show that physiologically tolerable concentrations of acetic acid can completely eradicate bacteria in mature biofilms *in vitro*. In addition, based on our clinical study, the treatment with acetic acid in combination with NPWT promotes wound healing. Acetic acid is presently one of the solely effective nontoxic treatment of biofilms in chronic infections, but further randomized controlled studies are needed to evaluate its clinical value.

KEY FINDINGS

- Acetic acid eradicates mature biofilms
- It is the acetic acid molecule itself in its nondissociated form that kills bacteria

INNOVATION

The usage of physiologically tolerable concentrations of acetic acid to eradicate bacteria in mature biofilms *in vitro* and promote wound healing in the clinic in combination with NPWT.

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AUTHOR DISCLOSURE AND GHOSTWRITING

The authors have nothing to disclose and no ghostwriting.

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Abbreviations and Acronyms

ESBL = extended-spectrum beta-lactamases
HCl = hydrochloric acid
LB = Luria broth
$NaHAc_2$ = sodium diacetate
NaOH = sodium hydroxide
NPWT = negative pressure wound therapy
MRSA = methicillin-resistant *Staphylococcus aureus*