journal of wound care JWC
VOLUME 29. NUMBER 5. MAY 2020

In vitro antimicrobial effects of chlorhexidine diacetate versus chlorhexidine free base dressings

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Objective: The aim of this study was to investigate the *in vitro* antimicrobial performance of a chlorhexidine diacetate dressing and a chlorhexidine free base dressing to determine if the free base form of chlorhexidine has the potential to be an effective alternative to the chlorhexidine salts used in conventional, chlorhexidine-based antimicrobial dressings.

Method: Dressing samples were inoculated with clinically relevant pathogenic microorganisms including Gram-positive and Gram-negative bacteria, yeasts and fungus, and subsequently evaluated for *in vitro* $\log_{10}$ reduction at 1-, 3-, and 7-day time points. Chlorhexidine mole content was also calculated as a function of dressing surface area for both sample types to allow for formulation-independent comparison between the dressings.

Results: The chlorhexidine free base dressing demonstrated >0.5 $\log_{10}$ superior mean antimicrobial efficacy at 67% of the experimental time points and equivalent mean antimicrobial efficacy (≤0.5 $\log_{10}$ different) at the remaining time points when compared with the chlorhexidine diacetate dressing. The chlorhexidine free base dressing was also found to contain 36% less chlorhexidine mole content than the chlorhexidine diacetate dressing.

Conclusion: Our results suggest that a dressing formulated with chlorhexidine free base can deliver *in vitro* antimicrobial performance at both a magnitude and rate that meets or exceeds that of a chlorhexidine diacetate-based dressing, while also allowing for a reduction in total chlorhexidine content per dressing. These findings could be of particular interest to researchers developing new antimicrobial technologies as well as to infection preventionists when evaluating antimicrobial products for use on clinical patients at elevated risk of infection.

Declaration of interest: The authors are employees of entrotech life sciences inc. which funded this project.

antimicrobial • chlorhexidine • *in vitro* • susceptibility • wound dressing

Intravascular (IV) catheters have a long history of medical use and an estimated 70% of patients receive an IV catheter during each hospital visit, totalling more than 300 million catheters in the US annually.[1,2] Preservation of wound and vascular access sites and the prevention of healthcare-associated infections (HAI), including catheter-related blood stream infections (CRBSI), are some of the greatest challenges faced by the health professionals who rely upon IV catheters to deliver patient therapies.[2–5] According to the US Centers for Disease Control and Prevention (CDC), there were an estimated 687,200 HAIs in US acute care hospitals in 2015 with approximately 11% of these infections resulting in patient death during hospitalisation.[6] A leading causes of HAIs and CRBSIs is a patient's own skin flora, and the emergence of deadly, drug-resistant microorganisms such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* spp. continues to increase the importance of infection prevention.[4,7] A particularly powerful tool in the fight to reduce HAI risk is the antimicrobial dressing, of which chlorhexidine-based antimicrobial dressings have become increasingly common.[7–11]

The synthesis and characterisation of pure chlorhexidine free base (CHX) were first reported in the 1950s.[12,13] While CHX was quickly identified as a powerful antimicrobial, it was found to exhibit poor solubility thus leading researchers to subsequently synthesise and investigate a variety of chlorhexidine salts created by reaction of CHX with a weak acid.[14] A few of these salts were found to exhibit improved solubility from CHX while also retaining some of its antimicrobial activity. By the early 1960s, health professionals had already begun using chlorhexidine digluconate (CHG) and chlorhexidine diacetate (CHA) solutions for surgical site disinfection.[15] Since then, a variety of chlorhexidine-based antimicrobial products have been developed in response to the global healthcare community's growing need for effective infection prevention tools.

While several chlorhexidine-based dressing technologies are currently available, they have historically been offered in a limited variety of salt formulations, concentrations, and configurations. As a result, the aim of this study was to quantify the time-dependent, *in vitro* antimicrobial performance of antimicrobial film dressings which use two fundamentally differing chlorhexidine formulations to determine if the free base form of chlorhexidine has the potential to be an effective alternative to the use of chlorhexidine salts in chlorhexidine-based antimicrobial

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Table 1. Pathogenic microorganisms used to challenge antimicrobial dressing test articles

Challenge microorganism species	Inoculum media	Plate media	Incubation temperature (°C)
Gram-positive bacteria			
Methicillin-resistant *Staphylococcus aureus* (MRSA), ATCC 33591	SCDB	SCDA	30–39
Methicillin-resistant *Staphylococcus epidermis* (MRSE), ATCC 51625	SCDB	SCDA	30–39
Multiple drug-resistant *Enterococcus faecium* (MDR), ATCC 51559	SCDB	SCDA	30–39
Vancomycin-resistant *Enterococcus faecalis* (VRE), ATCC 51299	SCDB	SCDA	30–39
Enterococcus faecium, ATCC 19434	SCDB	SCDA	35–39
Gram-negative bacteria			
Pseudomonas aeruginosa, ATCC 9027	SCDB	SCDA	35–39
Escherichia coli, ATCC 8739	SCDB	SCDA	35–39
Serratia marcescens, ATCC 8100	SCDB	SCDA	30–35
Yeasts			
Candida albicans, ATCC 10231	SDEX	SDEX	20–25
Candida parapsilosis, ATCC 14054	SDEX	SDEX	20–25
Candida tropicalis, ATCC 750	SDEX	SDEX	20–25
Fungus			
Aspergillus brasiliensis, ATCC 16404	SDEX	SDEX	20–25

ATCC—American type culture collection; SCDA—soybean casein digest agar; SCDB—soybean casein digest broth; SDEX—sabouraud dextrose agar

dressings. It was the expectation of the authors that clear, quantitative information reporting any differences in magnitude, rate of action, and/or overall *in vitro* antimicrobial performance of dressings using different chlorhexidine formulations would be of benefit to health professionals when determining the planned course of infection prevention treatment for patients.

Materials and methods

Description of sample test articles

The antimicrobial transparent thin film dressings evaluated in this study were the SurgiClear dressing, Ref. No. TWBD1019, Lot: 120326-300 (Covalon Technologies Ltd., US) and the PrevahexCHX dressing, Ref. No. 1485CH, Lot: 100068-001/2/3 (entrotech life sciences inc., US). These two dressings were selected for direct comparison because they were understood to be the most recently commercialised adhesive film dressings using different chlorhexidine formulations available at the time of this study. Both dressings are constructed of a non-antimicrobial polyurethane backing film coated on one side with an antimicrobial, pressure-sensitive adhesive (i.e., chemically non-reactive, self-adhesive) film capable of rapidly bonding to another surface, such as skin or an IV catheter, upon direct contact.

The SurgiClear dressing uses an adhesive which is impregnated with 3% wt/wt CHA ($C_{22}H_{30}C_{l2}N_{10}$. $2C_2H_4O_2$, 625.55g/mol, CAS No. 56-95-1) and 0.5% silver salts.[16] A published patent application from Covalon Technologies Ltd. (L. Yang and V. DiTizio, 27 December, 2012, United States Patent Office) indicated that the adhesive of this CHA-based dressing is likely composed of a polydimethylsiloxane-based silicone polymer film with a density of approximately 0.97g/cm^3. Additionally, published marketing materials from the manufacturer of the CHA dressing indicated the adhesive film of each 4x4cm dressing contains 7.2mg CHA,[17] which corresponds to approximate adhesive film concentration and thickness values of 0.45mg CHA/cm^2 and 0.0061", respectively.

The PrevahexCHX dressing uses an adhesive which is impregnated with 10% wt./wt. CHX ($C_{22}H_{30}C_{l2}N_{10}$, 505.45g/mol, CAS No. 55-56-1).[18] The CHX-based adhesive is composed of an acrylic-based polymer film with approximate density and thickness of 0.98g/cm^3 and 0.0011", respectively, as well as a chlorhexidine concentration of 0.27mg CHX/cm^2.

Samples of a non-antimicrobial, sterile polymer cover film were also included in this study as a negative control test article since antimicrobial-free versions of the CHX- and CHA-based dressings were unavailable. The sterile cover film also allowed for viability confirmation of the microbiological test system as the experimental protocol required that the film be maintained in direct, continuous contact with each

dressing test article during incubation to ensure uniform inoculum contact and minimise desiccation.

Regulatory compliance of microbiological testing activities

The microbiological testing activities described here were performed by an independent contract laboratory in compliance with the provisions of the US Environmental Protection Agency's (EPA) Good Laboratory Practices (GLP) regulations specified in Title 40 of the United States Code of Federal Regulations (CFR) Part 160.

In vitro $\log_{10}$ reduction method

All test articles were evaluated for *in vitro* $\log_{10}$ reduction (or *in vitro* kill time) in accordance with the protocols described in the Japanese Industrial Standard JIS Z 2801: 2010 (E) 'Antimicrobial products – test for antimicrobial activity and efficacy' and the International Organization for Standardization ISO 22196: 2011 'Measurement of antibacterial activity on plastics and other non-porous surfaces.' A brief summary of the laboratory test methods used in this study is provided, as more comprehensive descriptions of these methods have been published elsewhere.[19,20]

The CHX- and CHA-based dressings as well as samples of a control, non-antimicrobial, sterile polymer cover film were evaluated for antimicrobial performance against 12 challenge microorganisms. The microorganisms were obtained from the American Type Culture Collection (ATCC) and included a broad spectrum of drug-resistant bacteria, Gram-positive and Gram-negative bacteria, yeasts and fungus, all known to cause HAIs.[3,21–23] Inoculum solutions were prepared from microorganism stock cultures which were diluted with an appropriate diluent(s), including physiological saline solution 0.9% and/or saline Tween 80, to a final inoculum concentration of not less than 1.0×10^6 colony forming units (CFU) per 0.4ml. A list of the microorganisms used as well as their corresponding ATCC identification numbers, classification and laboratory culture conditions are presented in Table 1.

Microbiological evaluation was initiated by inoculation of each sample article (50mm x 50mm) with 0.4ml of microorganism solution before covering with a cover film (40mm x 40mm). Sample articles were then incubated for the control 0-hour and experimental 1-, 3- and 7-day time durations before neutralisation with Letheen Broth (LETH). For the evaluation, three replicate samples of the CHA-based dressing and negative control cover film were used at each experimental time point as both types were constructed of conventional materials and/or antimicrobial technologies. There were nine replicate sample articles used for the evaluation of the CHX-based dressing due to its novel and previously uncharacterised, antimicrobial dressing formulation. The experimental time points were selected to span the recommended clinical use range for a single dressing in accordance with the CDC's 2011 clinical practice guidelines for maintenance of vascular access devices.[4] LETH neutraliser was used due to its well-established history as an effective neutraliser of chlorhexidine, and its efficiency was experimentally determined in this study to be 70% or greater against the antimicrobial test articles for each microorganism. Following neutralisation, the microorganism population from each sample article was individually extracted, plated and incubated on growth media across a range of serial dilutions. Microorganism colonies were then manually counted on the serial dilution plate corresponding to a total population of 25–250 CFU per plate for bacteria and yeasts and 8–80 CFU per plate for fungus, to the maximum extent experimentally feasible. The mean total microorganism population was then calculated for each dressing type, time point and microorganism parameter combination.

Calculation of $\log_{10}$ reduction values

Microbial $\log_{10}$ reduction values were calculated by subtracting the logarithm of the arithmetic mean number of organisms recovered from the inoculated, treated article after exposure for the desired contact period from the logarithm of the arithmetic mean number of organisms recovered from the inoculated untreated control at 0-hour.

Statistical evaluation of $\log_{10}$ reduction values

An antimicrobial performance benchmark of 4.0 $\log_{10}$ reduction was defined as the minimum threshold for substantial antimicrobial dressing efficacy, consistent with practices commonly enforced by some governmental medical device regulatory agencies. It should be noted that the constituent microorganisms of a given species/strain population typically exhibit a significant level of intrinsic variability in their robustness and viability during the normal course of laboratory culture and propagation. This inherent characteristic of microbial populations can present limitations to the practical use of Mann-Whitney U tests and the associated p-values when comparing different sets of mean microorganism population counts. As a result, it is common in microbiological laboratory studies to define the statistical significance between mean population count values using alternative tools, such as pre-established, fixed tolerance values defined by the academic institutions and non-governmental organisations that create and standardise the microbiological testing protocols being used. Therefore, in this laboratory study, the fixed tolerance value of ±0.5 $\log_{10}$ described in the 'Criteria for antimicrobial effectiveness' section of the methodologically-similar US Pharmacopeia USP <51> antimicrobial effectiveness testing method was adopted to allow for the establishment of a practical threshold for the comparison between numerical results of mean microbiological population count values.[24] Thus, the

antimicrobial performance of two different sample types was considered to be statistically equivalent if their mean log_{10} reduction values were separated by 0.5 log_{10} or less, and statistically non-equivalent if separated by more than 0.5 log_{10} for a given time point and microorganism. In circumstances where the incubated organism population was experimentally 'too numerous to count' (TNTC), data points were treated as log_{10} reduction values of '0.00' for data analysis purposes since the 'TNTC' outcome indicates no organism reduction and thus an approximation of '0.00' is likely to be a conservative estimate.

Calculation of chlorhexidine mole content of dressing adhesive film as a function of surface area

The total chlorhexidine mole content of the dressing adhesive films was calculated as a function of surface area to allow for a direct, quantitative comparison between the dressings of different formulation, shape, thickness and size. The total chlorhexidine mole content of each dressing was calculated by division of chlorhexidine mass per unit surface area of a given dressing adhesive film by the molar weight of its chlorhexidine constituent as formulated.

Results

In vitro log_{10} reduction at 1-, 3- and 7-days

As presented in Fig 1, at the 1-day time point the CHX-based dressing demonstrated log_{10} reduction mean values ranging from 5.11–6.49 against all 11 drug-resistant bacteria, Gram-positive and Gram-negative bacteria and yeast organisms. While the CHA-based dressing achieved mean log_{10} reduction values ranging from 5.10–6.49 against the three Gram-negative bacteria species, it exhibited organism reductions below the 4.0 log_{10} performance benchmark for each of the remaining nine drug-resistant bacteria, Gram-positive bacteria and yeast species. Neither dressing achieved a ≥4.0 log_{10} reduction mean against the *Aspergillus brasiliensis* fungus organism. Lastly, the negative control cover film demonstrated log_{10} reduction mean values ranging from –1.22 to +2.96 against all 12 organisms.

At the 3-day time point, the CHX-based dressing demonstrated log_{10} reduction mean values in the range of 5.63–6.93 log_{10} against the 11 drug-resistant bacteria, Gram-positive and Gram-negative bacteria and yeast species. The CHA-based dressing demonstrated improved antimicrobial performance by achieving mean log_{10} reductions, ranging from 4.43–6.93 log_{10}

Fig 1. Summary of the mean *in vitro* log_{10} reduction values of the two antimicrobial dressing types and the polymer cover film control at 1-, 3-, and 7-day time points. Colour-temperature indicates magnitude of microbial log_{10} reduction observed. Mean *in vitro* log_{10} reduction values ≥4.0 log_{10} are presented in shades of green which increase in darkness with magnitude, while log_{10} reduction values <4.0 log_{10} transition from shades of light yellow to red with decreasing magnitude. *Actual experimental value was 'too numerous to count' (TNTC) and is thus presented here as '0.00' for data analysis purposes since the TNTC outcome indicates no organism reduction and the approximation of this value as '0.00' is likely to be a conservative estimate for the actual value

Challenge organism		Starting titer (CFU/sample)	Cover film negative control mean log_{10} reduction				PrevahexCHX mean log_{10} reduction			SurgiClear mean log_{10} reduction			Mean log_{10} reduction colour temperature scale
			0-Hour	1-Day	3-Day	7-Day	1-Day	3-Day	7-Day	1-Day	3-Day	7-Day	
Gram (+) Bacteria	MRSA	2.4×10^6	0	0.66	1.51	2.65	6.05	6.18	6.38	3.56	4.43	2.76	
	MRSE	1.3×10^6	-0.18	2.96	1.71	1.97	5.93	6.02	6.1	3.52	5.12	6.1	-2
	E. faecium (MDR)	1.1×10^6	-0.04	-0.11	2.41	2.55	6.09	5.63	5.67	2.66	4.69	3.21	-1
	E. faecalis (VRE)	1.8×10^6	0	-0.09	4.01	2.4	6.24	6.21	6.44	2.77	4.72	6.44	0
	E. faecium	4.3×10^6	-0.01	1.16	2.94	3.71	6.42	6.07	6.55	3.11	6	6.51	1
Gram (-) Bacteria	*P. aeruginosa*	2.0×10^6	-0.05	-1.22	-0.92	1.48	6.3	6.26	6.3	5.21	6.3	6.17	2
	E. coli	4.3×10^6	0.01	-0.79	-0.13	3.86	6.22	6.64	6.64	6.64	6.64	6.64	3
	S. marcescens	8.6×10^6	-0.01	2.89	4.63	4.63	6.28	6.93	6.46	5.1	6.93	6.93	4
Yeasts	*C. albicans*	1.2×10^6	-0.04	0.14	1.85	3.77	6.07	6.07	6.07	2.23	2.74	6.07	5
	C. parapsilosis	3.3×10^6	-0.04	-0.04	-0.21	-0.12	5.11	5.91	5.79	0*	1.64	2.73	6
	C. tropicalis	4.7×10^6	0.03	0.17	1.26	1.31	6.49	6.58	6.5	2.69	3.32	3.95	7
Fungus	*A. brasiliensis*	3.7×10^6	0.04	0.41	0.46	0.5	2.99	3.89	3.75	1.72	1.67	1.74	

against all eight of the drug-resistant, Gram-positive, and Gram-negative bacteria species, but it continued to produce mean $\log_{10}$ reductions below the 4.0 $\log_{10}$ performance benchmark against all of the yeast organisms. Like the observations at the 1-day point, neither of the antimicrobial dressings produced a ≥4.0 $\log_{10}$ reduction mean against *Aspergillus brasiliensis*. The negative control cover film produced bacterial $\log_{10}$ mean reductions of 4.01 and 4.63 against vancomycin-resistant *Enterococcus faecalis* (VRE) and *Serratia marcescens*, respectively, and $\log_{10}$ reduction mean values ranging between –0.93 to +2.94 $\log_{10}$ against the remaining 10 microorganisms.

At the 7-day experimental time point, the CHX-based dressing produced $\log_{10}$ reduction mean values ranging between 5.67–6.64 against the drug-resistant bacteria, Gram-positive and Gram-negative bacteria and yeast microorganisms. The CHA-based dressing produced $\log_{10}$ reduction mean values ranging from 6.10–6.93 against seven of the drug-resistant, Gram-positive and Gram-negative bacteria and yeast organisms. While it produced an improved performance of 6.07 $\log_{10}$ reduction mean against *Candida albicans* at this time point, the CHA-based dressing also demonstrated a decrease in antimicrobial performance below the mean 4.0 $\log_{10}$ reduction threshold against MRSA and VRE. The CHA-based dressing had not demonstrated ≥4.0 $\log_{10}$ reduction against the *Candida tropicalis* and *Candida parapsilosis* yeast species by the 7-day time point. Both antimicrobial dressings failed to achieve a ≥4.0 $\log_{10}$ reduction mean against the fungus challenge microorganism. Lastly, the cover film control demonstrated a mean bacterial reduction of 4.63 $\log_{10}$ against *Serratia marcescens*, while producing reduction mean values below the 4.0 $\log_{10}$ benchmark for the remaining 11 microorganisms.

Dressing chlorhexidine mole content as a function of dressing surface area

The total chlorhexidine mole content of the CHA-based dressing was determined to be 7.2×10^{-7} mol chlorhexidine/cm^2, and the total chlorhexidine mole content of the CHX-based dressing was found to be 5.3×10^{-7} mol chlorhexidine/cm^2. This result indicated that the total chlorhexidine mole content of the CHA-based dressing was 1.36 times that of CHX-based dressing, as a function of dressing surface area.

Discussion

The CHX-based dressing was observed to demonstrate *in vitro* antimicrobial performance superior (>0.5 $\log_{10}$ greater mean) to the CHA-based dressing at 67% of the 36 total experimental data points, and equivalent antimicrobial performance (≤0.5 $\log_{10}$ different mean) at the remaining data points. Furthermore, the CHA-based dressing did not demonstrate superior antimicrobial performance to the CHX-based dressing at any of the 36 data points. This finding is counterintuitive as the CHA-based dressing also contains silver salts, while CHX-based dressing contains a single antimicrobial agent. Both the CHX- and CHA-based antimicrobial dressings demonstrated statistically superior *in vitro* antimicrobial performance versus the negative control cover film against the challenge microorganisms for 100% and 94% of the experimental time points, respectively. No challenge microorganisms used in this study were obligate aerobes and thus the reported *in vitro* performance values were not expected to be significantly impacted by the presence of cover films.

The total chlorhexidine mole content of both dressings was determined to identify the cause of the observed differences in their *in vitro* antimicrobial performance. Specifically, chlorhexidine mole content of the dressings was calculated as a function of adhesive surface area to allow for an accurate side-by-side, quantitative comparison of the dressings regardless of shape or size. The total chlorhexidine mole content of the 3% wt/wt CHA-based dressing was determined to be 36% greater than the total chlorhexidine mole content of an equivalently sized sample of the 10% wt/wt CHX-based dressing. This finding was unexpected considering differences in antimicrobial performance of the dressings as reported above, in part because the adhesive of the CHA-based dressing also included 0.5% wt/wt silver salts which were incorporated by the manufacturer for the stated purpose of enhancing efficacy against Gram-negative bacteria,[17] and also because of the recently published study by Szweda et al. which reported several different silver-based antimicrobial dressings produce substantial *in vitro* antimicrobial efficacy within 24 hours.[25] However, the patent application for the CHA-based dressing adhesive formulations includes cationic triarylmethane dye-photo-stabilised silver salts which may include silver nitrate, silver acetate and/or silver acetate. The possible inclusion of a cationic triarylmethane dye with a silver salt(s) adds significant complexity to predicting the antimicrobial performance contribution of the silver salt(s) as this class of organic aromatic dye species may interact with neighbouring chlorhexidine species within the CHA-containing adhesive matrix. Additionally, the silver salts referenced above are each composed of anionic species capable of forming stable, water-soluble salts with chlorhexidine, thus further adding to the possibility of additional complex intermolecular interactions between the chlorhexidine and silver species within the CHA-based adhesive.[14] Therefore, the observed differences in the *in vitro* antimicrobial performance between the two dressing types are not directly attributable only to simple differences in chlorhexidine mole content or the presence of silver salts in the CHA-based dressing. Alternatively, it is likely that the unique and complex combination of chlorhexidine formulation, chlorhexidine concentration, film thickness and polymer matrix composition of each dressing may collectively be the primary driving force behind the observed differences in their antimicrobial performance,

in a manner consistent with the Higuchi kinetic model for drug release from a thin ointment film (i.e., polymer matrix in this case). In this model, the rate of drug release from a film reservoir is a time-dependent function affected by film thickness, drug solubility, initial drug concentration and a system-specific diffusion coefficient, and each of these corresponding parameters for the two dressings studied are either known to have, or would be reasonably expected to have, different values. The hypothesis that the two dressings release chlorhexidine in a manner consistent with Higuchi kinetics is supported by a technical bulletin available from the CHA-dressing manufacturer which states, '... chlorhexidine diacetate present in IV Clear is less easily removed [than CHG] from the dressing because of its lower water solubility relative to chlorhexidine digluconate. In addition, the majority of the chlorhexidine diacetate is embedded within a hydrophobic silicone matrix, which helps to moderate the release of chlorhexidine...'.[17] Therefore, while differences in chlorhexidine formulation likely play a contributing role in the observed disparity in speed and magnitude of *in vitro* antimicrobial performance between the two dressings, the results of this microbiological investigation are not sufficiently comprehensive to allow for the quantification of this formulation-dependent contribution in isolation due to the presence of additional and uncontrolled chemical, physical and compositional differences between the adhesive films. Nonetheless, the results of this study clearly demonstrate that a dressing formulated with CHX can provide *in vitro* antimicrobial performance similar, or in some cases superior, to a dressing formulated with a conventional chlorhexidine salt, such as CHA.

Limitations

While this study was designed to investigate the antimicrobial feasibility of a CHX-based dressing compared with a CHA-based dressing, it is important to note that microbial kill is one of several collectively important attributes that must be considered in the selection of an antimicrobial substance and corresponding formulation for a dressing. Furthermore, there is currently a scarcity of published clinical research on the general tolerability and patient risk profile for CHX relative to that of more common chlorhexidine salt formulations. Historically, research investigations of the biocompatibility and patient tolerance of chlorhexidine-based antimicrobial products have been predominantly focused on the investigation of CHG dressings and CHG/alcohol solutions formulated within the limited range of commercially available product formulations.[9,26] The results of these studies indicate that, despite being generally well-tolerated by a significant majority of clinical populations, a small but measurable number of patients experience skin sensitivity and allergological issues following exposure to chlorhexidine salts. As a result, while the findings of this study demonstrate the *in vitro* antimicrobial performance of CHX, they also highlight the need for future side-by-side comparative investigations of not only antimicrobial efficacy but also of patient tolerability and safety as a function of chlorhexidine formulation.

While a hypothetical explanation for the observed differences in antimicrobial performance of the CHX- and CHA-based dressings is postulated above, additional microbiological, chemical analysis and diffusion rate studies would be necessary to definitively identify the origin(s) of this phenomenon. Additionally, it should be noted that a limitation of the $\log_{10}$ reduction data presented here is that these data were generated in a GLP-compliant *in vitro* laboratory study, thus these findings do not necessarily predict clinical efficacy of the antimicrobial dressing products evaluated.

Conclusions

An *in vitro* antimicrobial performance study was conducted on dressings with two different types of chlorhexidine formulations at multiple experimental time points, against a broad spectrum of clinically-relevant, pathogenic microorganisms. The CHX-based dressing demonstrated *in vitro* antimicrobial performance superior to the CHA- and silver-based dressing at 67% of experimental time points and equivalent antimicrobial efficacy at the remaining time points. The CHX-based dressing was also observed to produce significant antimicrobial efficacy at a more rapid rate than the CHA- and silver-based dressing, with the largest difference in magnitude and spectrum of antimicrobial efficacy occurring at the 1-day time point. This finding could be of particular interest when evaluating antimicrobial products for clinical use.

Additionally, the 10% wt/wt CHX-based dressing was determined to contain 36% less total chlorhexidine mole content than a 3% wt/wt CHA- and silver-based dressing of equivalent size due to differences in the antimicrobial adhesive film thicknesses of the dressings. This finding suggests that a dressing formulated with CHX can deliver *in vitro* antimicrobial performance at both a magnitude and rate that meet or exceed the performance of a CHA-based comparator dressing while also allowing for a reduction in total chlorhexidine mole content per dressing. However, the experimental data generated in this study are not sufficiently comprehensive to allow for the isolation and quantification of the contribution of chlorhexidine moiety formulation to the overall observed *in vitro* antimicrobial performance for the two dressings due to their additional differences in chlorhexidine concentration, presence or absence of sliver salts, polymer composition, and thickness of their adhesive films. Lastly, the *in vitro* data presented here supports the hypothesis that use of pure CHX in antimicrobial medical devices may provide a more rapid and effective alternative to the conventional use of chlorhexidine. JWC

Reflective questions

- Why is chlorhexidine commonly formulated as a salt in healthcare products?
- Would dressing adhesive composition or chlorhexidine formulation be expected to have a greater effect on antimicrobial efficacy?
- What environmental factors at a wound site might impact the speed and efficacy of different chlorhexidine formulations?

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