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Hydrogen-peroxide generating electrochemical bandage is active *in vitro* against mono- and dual-species biofilms

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ABSTRACT

Biofilms formed by antibiotic-resistant bacteria in wound beds present unique challenges in terms of treating chronic wound infections; biofilms formed by one or more than one bacterial species are often involved. In this work, the *in vitro* anti-biofilm activity of a novel electrochemical bandage (e-bandage) composed of carbon fabric and controlled by a wearable potentiostat, designed to continuously deliver low amounts of hydrogen peroxide (H_2O_2) was evaluated against 34 mono-species and 12 dual-species membrane bacterial biofilms formed by *Staphylococcus aureus*, *S. epidermidis*, *Enterococcus faecium*, *E. faecalis*, *Streptococcus mutans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Cutibacterium acnes*, and *Bacteroides fragilis*. Biofilms were grown on polycarbonate membranes placed atop agar plates. An e-bandage, which electrochemically reduces dissolved oxygen to H_2O_2 when polarized at −0.6 $V_{Ag/AgCl}$, was then placed atop each membrane biofilm and polarized continuously for 12, 24, and 48 h using a wearable potentiostat. Time-dependent decreases in viable CFU counts of all mono- and dual-species biofilms were observed after e-bandage treatment. 48 h of e-bandage treatment resulted in an average reduction of 8.17 ± 0.40 and 7.99 ± 0.32 $\log_{10}$ CFU/cm^2 for mono- and dual-species biofilms, respectively. Results suggest that the described H_2O_2 producing e-bandage can reduce *in vitro* viable cell counts of biofilms grown either in mono- or dual-species forms, and should be further developed as a potential antibiotic-free treatment strategy for treating chronic wound infections.

1. Introduction

Chronic wounds and associated infections are complex. In the United States, an estimated 6.5 million patients a year are affected by chronic wounds, with treatment costing ~$25 billion per year [1,2]. Wound infections can be recalcitrant to conventional antibiotic treatment [3,4]. The healing process involves several stages, including homeostasis, inflammation, granulation, and finally tissue remodeling [5]. Biofilms in wound beds may delay wound healing by one or more mechanisms, including decreasing the ability of fibroblasts and other cells to reach the wound site, impairing cellular communication, and triggering excessive inflammatory responses [6,7]. Biofilms in wounds often contain one or more species of bacteria and/or fungi. Microorganisms found in biofilms excrete extracellular polymeric substance (EPS), composed of glycopeptides, proteins, and/or extracellular DNA [8,9]. Limited availability of nutrients, low oxygen availability, low pH, and reduced water activity result in bacterial cells in inner layers of biofilms growing slowly, thereby becoming 'dormant', contributing to antibiotic tolerance [10,11]. As a result of the low metabolic activity of 'dormant' cells, antibiotics that depend on bacterial cellular activity are rendered poorly active, potentially enhancing selection of antibiotic resistance [12]. Accordingly, alternative approaches are needed to treat chronic wound infections.

Biocides and topical antimicrobials, such as phenols, formaldehyde, chlorhexidine gluconate, povidone iodine, alcohols, hydrogen peroxide (H_2O_2), medicinal honey, and hypochlorous acid (HOCl), are used for wound cleaning and debridement [13]. As with antibiotics, biofilms in wound beds can reduce the activity of biocides. Among these, there is

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particular interest in H_2O_2 and HOCl, natural biocides found in wound beds, produced as part of the cellular inflammatory response in wounds, albeit in low concentrations. H_2O_2 can improve wound healing [14,15]. H_2O_2 production by host immune cells improves migration of endothelial cells, keratinocytes and fibroblasts, and augments differentiation of keratinocytes, promoting wound healing [16,17]. Wound dressings containing such biocides are, however, not practical due to dissipation of the active substances over time. A wound-dressing system that continuously produces/delivers low amounts of H_2O_2 (or HOCl) to wound beds, could offer a therapeutic option for wound infections.

Previously, a novel electrochemical scaffold (e-scaffold) system composed of carbon fabric, two carbon-fabric electrodes and a reference electrode, was designed and developed to deliver controlled amounts of H_2O_2 (or HOCl) [18,19]. Anti-biofilm activity of H_2O_2- (and HOCl-) generating e-scaffolds was shown against bacterial and fungal mono-species and tri-species bacterial biofilms [20,21]. The e-scaffolds operated while immersed in a liquid electrolyte and required an external reference electrode, alongside a bench-top potentiostat for operation, prohibiting *in vivo* use. Accordingly, the H_2O_2-generating e-scaffold was transformed to a H_2O_2-generating electrochemical bandage (e-bandage) designed to be placed atop infected wounds, and operated using a wearable potentiostat with a hydrogel electrolyte (instead of requiring liquid immersion) [22]. Earlier, operational principles and electrochemistry of the H_2O_2-generating e-bandage, and design and characterization of the wearable potentiostat were described, with proof of concept anti-biofilm activity demonstrated against *Acinetobacter baumannii* biofilms in an agar wound biofilm model which mimics a wound bed environment [22,23]. To advance the e-bandage towards *in vivo* application, *in vitro* activity against mono- and dual-species biofilms of 34 bacterial isolates and 12 dual-species biofilms was tested. The dual-species biofilms combinations were selected based on the frequency with which these bacterial species are associated with polymicrobial wound infections [24].

2. Methods and materials

Electrochemical bandage: The e-bandage and wearable potentiostat are described in a previous study [22]. Briefly, the e-bandage is comprised of three electrodes embedded in a bandage-like structure: a working electrode and counter electrode made up of circular conductive carbon fabric patch having an area of 1.77 cm^2 (Panex 30 PW-06, Zoltek Companies Inc., St. Louis, MO), and a silver/silver chloride (Ag/AgCl) wire which acts as quasi reference electrode (QRE). The working electrode potential is controlled at −0.6 $V_{Ag/AgCl}$ using a wearable potentiostat. H_2O_2 is generated on the working electrode by O_2 reduction. Two cotton fabric layers separate the working and counter electrode, with an additional carbon fabric layer used above the counter electrode to enhance moisture retention. Fabrics are attached using silicone adhesive, which partially covers the outer edge of the electrodes and cotton fabric layers. The QRE is glued between the two cotton fabric layers, separating the carbon electrodes. Titanium wires (TEMCo, Amazon.com, catalog #RW0524) are attached to flanking ends of the e-bandage via nylon sew-on caps (Dritz, Spartanburg, SC, item#85). E-bandages are steam sterilized in autoclave at 121 °C for 20 min.

For each e-bandage treatment experiment, sterile e-bandages were pre-hydrated for 15 min in sterile 1 × phosphate buffer saline (1 × PBS) in a Petri dish. 1.8% w/v sterile hydrogel was prepared by mixing autoclaved xanthan gum (Namaste Foods LLC, Coeur d'Alene, ID) in 1 × PBS. Prior to starting e-bandage treatment, sterile hydrogel was added to the fabric layers of e-bandage and to the top of the membrane biofilm, as described previously [22].

***In vitro* agar membrane mono-species and dual-species biofilms:** Table 1 shows the bacteria studied. For mono-species biofilms, a single colony of freshly streaked bacteria growing on tryptic soy or sheep blood agar (refer to Table S1) was added to a test tube containing 3 ml of tryptic soy broth (TSB) or brain heart infusion (BHI) broth supplemented

Table 1
Bacterial isolates and their characteristics.

Bacteria	Isolate Designation	Isolate Characteristics	Starting Inoculum for Mono-species Biofilms
Staphylococcus aureus	USA100	Clinical isolate, resistant to methicillin	2.5 μl of 0.5 McFarland growth tube
S. aureus	USA200	Clinical isolate, resistant to methicillin	2.5 μl of 0.5 McFarland growth tube
S. aureus	USA300	Clinical isolate, resistant to methicillin	2.5 μl of 0.5 McFarland growth tube
S. aureus	IDRL-6169	Periprosthetic hip isolate; resistant to methicillin and mupirocin	2.5 μl of 0.5 McFarland growth tube
S. aureus	Xen 30	Clinical isolate; resistant to methicillin	2.5 μl of 0.5 McFarland growth tube
S. aureus	IDRL-4284	Clinical isolate; resistant to methicillin	2.5 μl of 0.5 McFarland growth tube
Staphylococcus epidermidis	ATCC 35984	Catheter sepsis isolate; resistant to methicillin	2.5 μl of 3.0 McFarland growth tube
S. epidermidis	IDRL-6461	Periprosthetic knee infection isolate; susceptible to methicillin	2.5 μl of 3.0 McFarland growth tube
S. epidermidis	Xen 43	Catheter isolate; susceptible to methicillin	2.5 μl of 3.0 McFarland growth tube
Enterococcus faecalis	ATCC 29212	Urine isolate	2.5 μl of 0.5 McFarland growth tube
E. faecalis	IDRL-8618	Periprosthetic hip infection isolate	2.5 μl of 1.0 McFarland growth tube
E. faecalis	IDRL-7107	Periprosthetic knee infection isolate	2.5 μl of 1.0 McFarland growth tube
E. faecalis	IDRL-12374	Periprosthetic hip isolate, resistant to vancomycin and levofloxacin	2.5 μl of 1.0 McFarland growth tube
E. faecium	IDRL-11790	Abscess isolate; resistant to vancomycin and penicillin, and susceptible to linezolid	2.5 μl of 0.5 McFarland growth tube
Escherichia coli	IDRL-10366	bla_{KPC}-positive isolate; resistant to ceftolozane/tazobactam, imipenem, meropenem, ertapenem, ceftriaxone and cefepime	2.5 μl of 0.5 McFarland growth tube
E. coli	IDRL-7029	Periprosthetic hip infection isolate	2.5 μl of 0.5 McFarland growth tube
E. coli	IDRL-6199	Periprosthetic knee infection isolate	2.5 μl of 0.5 McFarland growth tube
E. coli	IDRL-8110	Blood isolate	2.5 μl of 0.5 McFarland growth tube
Pseudomonas aeruginosa	IDRL-7262	Periprosthetic hip infection isolate	2.5 μl of 10^4 CFU/ml growth tube
P. aeruginosa	Derived from ATCC 19660; (Xen 5)	Blood isolate	2.5 μl of 10^4 CFU/ml growth tube
P. aeruginosa	PAO1, ATCC 47085	Wound isolate; type strain	2.5 μl of 10^4 CFU/ml growth tube
P. aeruginosa	PA14	Wild type lab strain	2.5 μl of 10^4 CFU/ml growth tube

(*continued on next page*)

Table 1 (*continued*)

Bacteria	Isolate Designation	Isolate Characteristics	Starting Inoculum for Mono-species Biofilms
P. aeruginosa	PA14 Δ*katAB*	*katA* and *katB* double-knockout of PA14	2.5 μl of 10^4 CFU/ml growth tube
P. aeruginosa	IDRL-11442	Groin isolate; resistant to piperacillin/tazobactam, cefepime, ceftazidime, meropenem, aztreonam, ciprofloxacin and levofloxacin and susceptible to colistin	2.5 μl of 10^4 CFU/ml growth tube
Acinetobacter baumannii	ATCC 17978	Meningitis isolate	2.5 μl of 0.5 McFarland growth tube
A. baumannii	ATCC BAA-1605	Sputum isolate; resistant to ceftazidime, gentamicin, ticarcillin, piperacillin, aztreonam, cefepime, ciprofloxacin, imipenem and meropenem	2.5 μl of 0.5 McFarland growth tube
A. baumannii	ARLG-1268	Wound isolate; resistant to amikacin, ampicillin, cefepime, ceftazidime, ciprofloxacin and tobramycin	2.5 μl of 0.5 McFarland growth tube
Klebsiella pneumoniae	IDRL-10377	bla_{KPC}-positive isolate; resistant to ceftolozane/tazobactam, imipenem, meropenem, ertapenem, ceftriaxone and cefepime	2.5 μl of 0.5 McFarland growth tube
Bacteroides fragilis	IDRL-11882	Periprosthetic knee infection isolate	2.5 μl of 2.0 McFarland growth tube
Cutibacterium acnes	IDRL-7676	Periprosthetic shoulder infection isolate	2.5 μl of 2.0 McFarland growth tube
C. acnes	IDRL-7751	Spine-implant infection isolate	2.5 μl of 2.0 McFarland growth tube
C. acnes	IDRL-7844	Spine-implant infection isolate	2.5 μl of 2.0 McFarland growth tube
Streptococcus mutans	IDRL-7131	Periprosthetic knee infection isolate	2.5 μl of 1.0 McFarland growth tube
S. mutans	IDRL-6249	Blood isolate	2.5 μl of 1.0 McFarland growth tube

with 1% glucose (refer to Table S1) and incubated at 37 °C under shaking conditions (120 rpm) for aerobic bacteria, at 37 °C in anaerobic jars (for *C. acnes* and *B. fragilis*), or at 37 °C in 5% CO_2 atmosphere (for *S. mutans*). Bacteria were grown until they reached McFarland standards or cell-densities shown in Table 1. 2.5 μl of freshly grown bacteria in broth was spotted onto the center of 13 mm sterile polycarbonate membranes (Whatman® Cat. No. 110406, GE Healthcare) that were placed on tryptic soy agar (TSA) or sheep blood agar plates. Bacterial spots were air dried; TSA plates (for aerobic bacteria) were incubated at 37 °C for 24 h for aerobic bacteria; sheep blood agar plates (for *C. acnes* and *B. fragilis*) were incubated at 37 °C in anaerobic jars for 48 h; and sheep blood agar plates (for *S. mutans*) were incubated at 37 °C in a 5% CO_2 atmosphere for 48 h. After incubation, polycarbonate membranes were moved onto new TSA plates. To establish mono-species biofilm, starting inocula were varied by bacterial strain in order to achieve ~8.00 to 8.5 $\log_{10}$ colony forming units (CFU)/cm^2.

To establish dual-species membrane biofilms, biofilms were targeted to harbor ~7.5 to 8.5 $\log_{10}$ colony forming units (CFU)/cm^2 of each isolate in the pair with relatively equal amounts of bacterial cells of each species. Details on the 12 dual-species membrane biofilm pairs studied, starting inocula, and selective growth media used post e-bandage treatment to quantify each component, are shown in Table S2.

Treatment of mono-species and dual-species membrane biofilms using e-bandages: E-bandages were placed atop membrane biofilms, hydrogel added as described above, and sterile Tegaderm™ applied to cover the surface of the e-bandage. The wearable potentiostat was connected to e-bandage electrodes and a 3 V battery inserted to start treatment. Biofilms were treated for 12, 24, and 48 h. Controls were biofilms exposed to non-polarized e-bandages (e-bandages not connected to a potentiostat). The potential of the working electrode relative to the QRE was measured at the start and end of each experiment. Additionally, for 48 h treatment experiments, the potential was measured and a new battery inserted into the wearable potentiostat after 24 h of polarization.

Biofilm quantification after e-bandage treatment: After treatment, both Tegaderm™ and e-bandages were removed from membrane biofilms. e-bandages were placed in sterile Petri dishes containing 5 ml of 1 × PBS. Surfaces of the e-bandages were gently scraped using sterile pipette tips to remove attached cells. The PBS solution and membrane biofilms were transferred to a sterile 15 ml Falcon tube, vortexed for 30 s, sonicated in a water bath for 5 min and vortexed again for 30 s. The suspension was centrifuged at 5000 rpm for 10 min and the supernatant discarded. 1 ml of 1 × PBS was added; 100 μl of this suspension was serially diluted (10-fold dilutions) in 1 × PBS and colony forming units (CFUs) determined by spread-plating 100 μl of each dilution tube onto sterile TSA or sheep blood agar plates (Table S1). TSA plates (aerobic bacteria) were incubated at 37 °C for 24 h; sheep blood agar plates (*C. acnes* and *B. fragilis*) were incubated at 37 °C in anaerobic jars for 48 h; and sheep blood agar plates (*S. mutans*) were incubated at 37 °C in 5% CO_2 atmosphere for 48 h; results were reported as CFU/cm^2. 100 μl of each undiluted suspension was added to a tube containing 5 ml of sterile TSB or BHI supplemented with 1% glucose (Table S1) and incubated at 37 °C for 24 h to check for potential bacterial growth. The limit of detection for the spread-plating method was considered 0.87 $\log_{10}$ CFU/cm^2 and that of broth culture 0.71 $\log_{10}$ CFU/cm^2.

Fig. S1 outlines the experimental process starting from sterilizing the polycarbonate membrane to quantifying biofilm bacteria after e-bandage treatment.

Statistical analysis: Descriptive summaries for each bacterial isolate by treatment group at 0, 12, 24 and 48 h are reported as mean ± standard deviation values in $\log_{10}$ CFU/cm^2. Comparisons across all experimental groups were first performed using Kruskall Wallis test. Further comparisons between groups in a pairwise manner were performed using the Wilcoxon rank sum test. Non-parametric tests were used due to small sample sizes and inability to support the assumption of normal distribution of the data. Analysis was performed for each bacterial isolate, and treatment time. All tests were 2 sided; p-values less than 0.05 were considered statistically significant. Analysis was performed using SAS software (version 9.4; SAS Institute). Graphs were generated in GraphPad Prism (software version 8.0, GraphPad Software). Each data value represents at least 3 replicates tested on different days.

3. Results

Mono-species biofilms: Exposure of bacterial biofilms to H_2O_2-producing e-bandages resulted in significant reductions ($p < 0.05$) in viable cells of biofilms of all isolates (Fig. 1). Time-dependent decreases in biofilm CFU were observed ($p < 0.05$). The mean reduction of mono-species biofilms after 12 h exposure to H_2O_2-producing e-bandages was 2.35 ± 0.92 $\log_{10}$ CFU/cm^2 ($p < 0.05$). The mean reduction of mono-species biofilms after 24 h exposure to H_2O_2-producing e-bandages was 5.13 ± 1.45 $\log_{10}$ CFU/cm^2 ($p < 0.05$). 48 h e-bandage treatment resulted in an average reduction of 8.17 ± 0.40 $\log_{10}$ CFU/cm^2 ($p < 0.05$). No colonies were observed on agar plates and no growth was

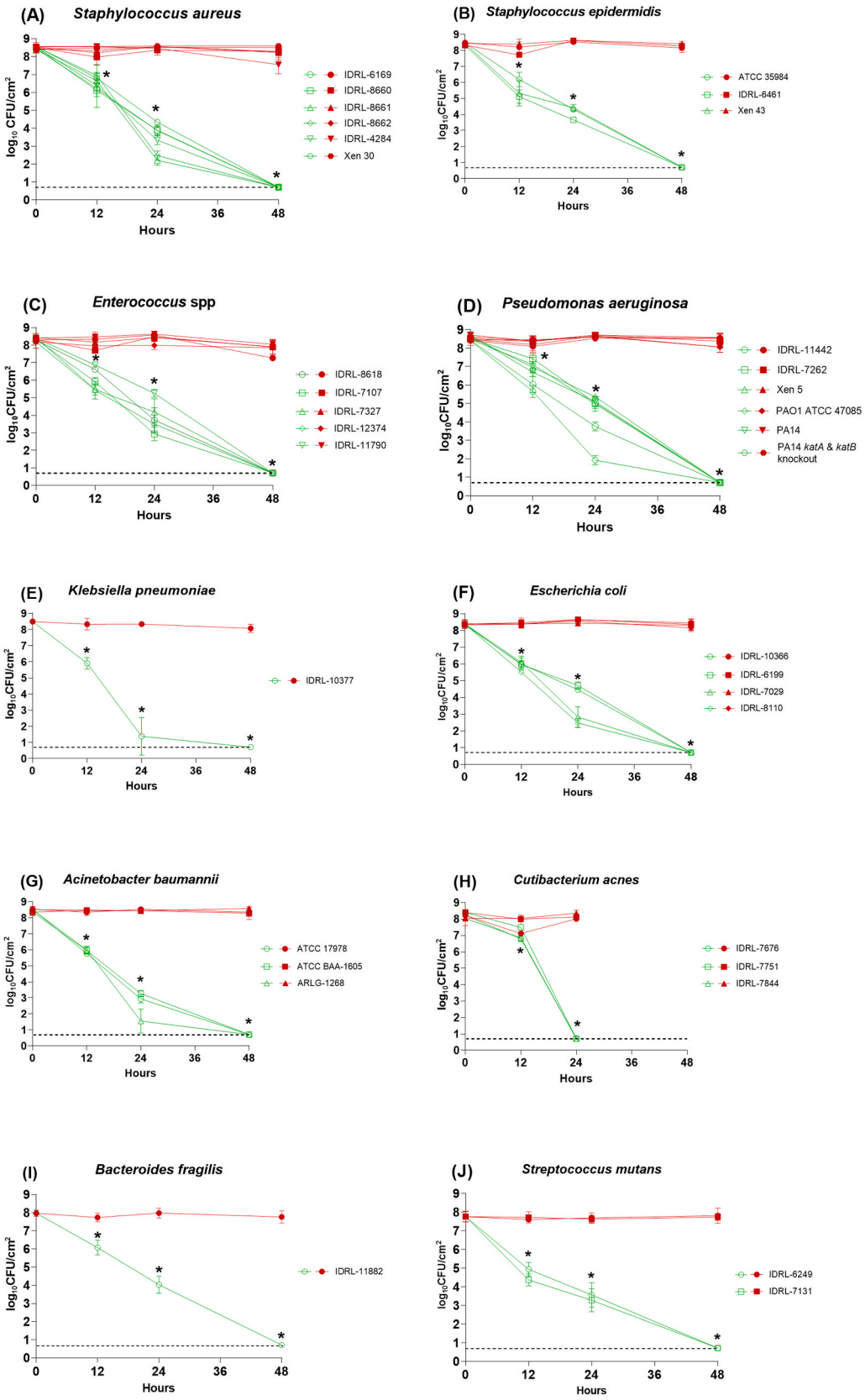

Fig. 1. E-bandage treatment of mono-species biofilms at 12, 24, and 48 h. Data points represent means and error bars represent standard deviation (n = 3). Data showing statistical significance (p value < 0.05) are denoted by (*) in the graphs. Red solid symbols represent the non-polarized (control) group and green open symbols represent the polarized (active treatment) group. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

observed in broth cultures after 48 h of e-bandage treatment. Gram-positive and Gram-negative mono-species biofilms were equally susceptible to e-bandage treatment at the exposure times studied; after 48 h of treatment, the average reduction in viable counts of Gram-positive mono-species biofilms was 8.09 ± 0.44 $\log_{10}$ CFU/cm^2 while that of Gram-negative mono-species biofilms was 8.14 ± 1.27 $\log_{10}$ CFU/cm^2. An outlier in the reduction trend was found with *C. acnes*, with earlier biofilm reductions. The average reduction of *C. acnes* biofilms was 4.37 ± 0.69 $\log_{10}$ CFU/cm^2 after 12 h of treatment ($p < 0.05$), and 8.16 ± 0.20 $\log_{10}$ CFU/cm^2 after 24 h of treatment ($p < 0.05$), with no growth on plates or in broth. An interesting result was observed with *P. aeruginosa* PA14 Δ*kat*AB (an isolate that lacks *katA* and *katB* catalase genes). The average biofilm reduction for this isolate after 24 h of e-bandage treatment was 6.59 ± 0.11 $\log_{10}$ CFU/cm^2, more than the average biofilm reduction of its wild type parent isolate *P. aeruginosa* PA14 (3.52 ± 0.12 $\log_{10}$ CFU/cm^2, Supplementary Fig. S2, $p < 0.05$).

Dual-species biofilms: Since clinically relevant chronic wound biofilms often harbor more than one species of bacteria, 12 dual-species biofilms were assessed (Table S2). As was the case with mono-species biofilm exposure, a time-dependent decrease in overall viable cell counts of biofilms was observed with exposure to H_2O_2-producing e-bandages (Fig. 2, $p < 0.05$). 12 h treatment resulted in mean reductions of 2.57 ± 0.49 $\log_{10}$ CFU/cm^2. Mean reductions of 4.10 ± 0.46 $\log_{10}$ CFU/cm^2 were observed in viable cell counts of dual-species biofilms when exposed to e-bandages for 24 h. 48 h of e-bandage exposure resulted in mean reductions of 7.99 ± 0.32 $\log_{10}$ CFU/cm^2 with no colonies on agar plates. No significant differences in average reductions of cell quantities of bacterial isolates when grown as mono-*versus* dual-species were found, except for *A. baumannii* ARLG-1268 and *K. pneumoniae* IDRL-10377. When their mono-species biofilms were treated for 24 h, the average viable cell reduction was higher than in dual-species biofilms (Supplementary Fig. S3; $p < 0.05$). When *A. baumannii* ARLG-1268 was grown alone, the mean reduction after 24 h of e-bandage treatment was 7.11 $\log_{10}$ ±1.07 $\log_{10}$ CFU/cm^2 whereas it was 4.51 ± 0.29 $\log_{10}$ CFU/cm^2 in dual-species biofilms (when grown with either *S. epidermidis* ATCC 35984 or *P. aeruginosa* IDRL-11442). For *K. pneumoniae* IDRL-10377, the average reduction after 24 h of treatment was 7.43 ± 1.53 $\log_{10}$ CFU/cm^2 when grown alone *versus* 3.89 ± 0.44 $\log_{10}$ CFU/cm^2 in dual-species biofilms (when grown with either *Escherichia coli* IDRL-10366 or *B. fragilis* IDRL-11882).

4. Discussion

This work describes the anti-biofilm activity of an H_2O_2-generating e-bandage with a wearable potentiostat. The e-bandage, which is designed to continuously produce low concentrations of H_2O_2 [22], was tested on membrane biofilms on agar surfaces to simulate application to wound biofilms. H_2O_2 is used clinically for wound cleaning and debridement. However, its rapid oxidation results in loss of activity over time when applied in bulk [25]; this limitation may be overcome by continuous production at low concentrations [18]. The e-bandage evaluated in the current study is powered by an inexpensive battery-operated wearable potentiostat. It is being designed to be directly applied to biofilm-harboring wounds. Previously, it was tested against a single mono-species *A. baumannii* biofilm [22]; here, it was tested against a wide array of mono- and dual-species bacterial biofilms.

Biofilms in wound beds impair wound healing of chronic wounds [6,7]. In this regard, improved and effective biofilm-targeted therapies, which augment wound healing, are needed. Multiple species of bacteria populate chronic wounds, and thus, to clinically recapitulate the clinical scenario, it is important that studies involving strategies to treat wound biofilm infections include polymicrobial biofilms [26,27]. Previously, the anti-biofilm activity of H_2O_2-producing e-scaffolds against *S. aureus*, *P. aeruginosa* and *A. baumannii* biofilms was demonstrated [18,20]. Subsequently, anti-biofilm activity of H_2O_2-producing e-scaffolds against tri-species biofilms of *S. aureus*, *P. aeruginosa* and *Candida albicans* was shown [21]. Time-dependent decreases in biofilm counts were observed. Together, these studies show that e-scaffolds can reduce both mono- and tri-species biofilms *in vitro*.

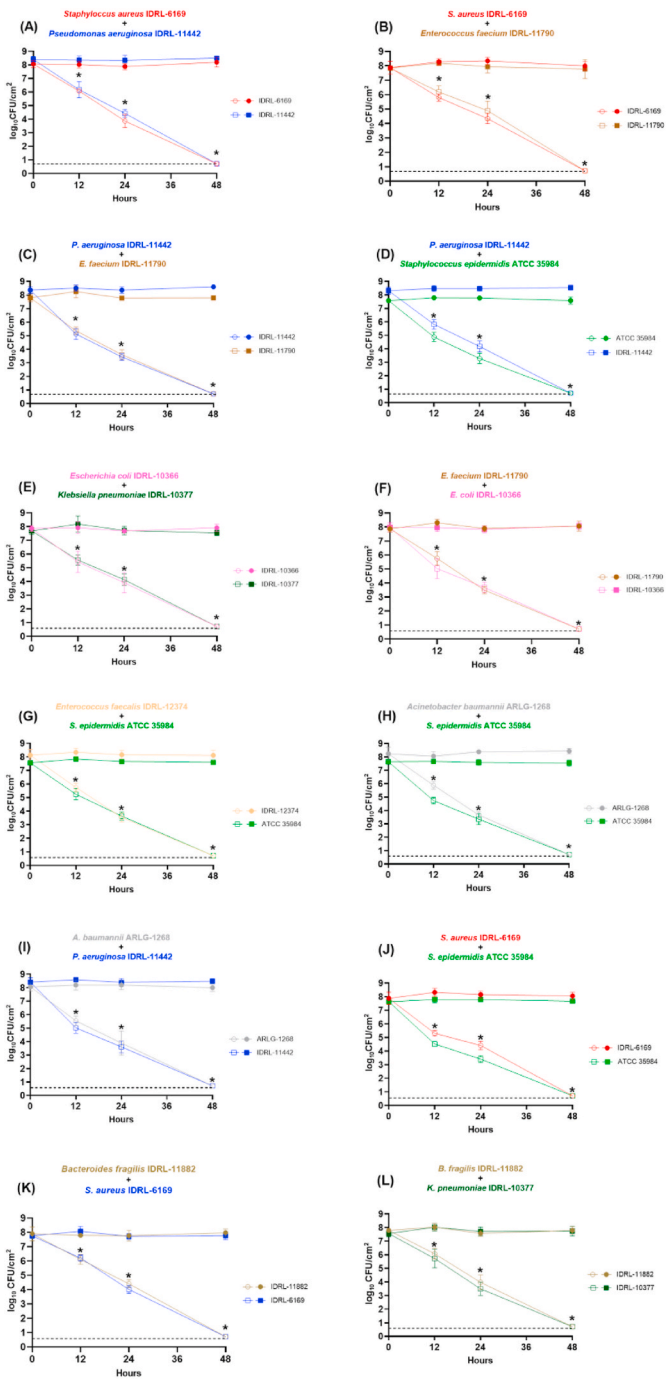

Fig. 2. E-bandage treatment of dual-species biofilms at 12, 24, and 48 h. Data points represent means and error bars represent standard deviation (n = 3). Data showing statistical significance (*p* value < 0.05) are denoted by (*) in the graphs. Solid symbols represent the non-polarized (control) group and open symbols represent the polarized (active treatment) group.

In this work, the anti-biofilm activity of a novel recently described e-bandage system against mono- and dual-species biofilms formed by a wide variety of bacteria was assessed. Species evaluated included *S. aureus*, *P. aeruginosa*, *S. epidermidis*, *Enterococcus faecium*, *Enterococcus faecalis*, *S. mutans*, *E. coli*, *K. pneumoniae*, *C. acnes*, and *B. fragilis*, which together represent many of the species found in wounds [28–32]. Results for the e-bandage treatment of mono-species biofilm show that the

H_2O_2-producing e-bandage reduced biofilms regardless of bacterial species. 12 dual-species biofilm combinations were selected for study (Table S2) based on a review of the literature and factoring in commonly found species in polymicrobial infections, wound biofilms formed by bacteria with high virulence, wound biofilms associated with traumatic injuries and biofilms formed by bacteria resistant to multiple antibiotics [33,34]. Treatment of mixed-species biofilms within wound-beds can be challenging in clinical settings. In such cases, antibiotic combination therapy comprising more than one class of antibiotics may be needed and the presence of more than one species of bacteria may, in and of itself, provide protection against antimicrobial strategies [35–39]. The most studied dual-species biofilm is that of *S. aureus* and *P. aeruginosa*. Mutual protective roles of these two in acute and chronic wound infections have been described [40,41]. In one study, the authors found that *C. albicans* with *S. aureus* and *P. aeruginosa* supported bacterial colonization and enhanced the resistance to an anti-fungal drug [38,42]. The results obtained in this work suggest that the described H_2O_2-producing e-bandage is active in reducing mono- and dual-species biofilms.

Among various biocides approved for clinical use for wound cleaning and debridement, H_2O_2 has been recognized for its rapid sterilization and disinfection properties, as a result of its ability to form reactive oxygen species (ROS). Bacterial cells present in biofilms produce enzymes such as catalase, superoxide dismutase, peroxidases, and reductases [43,44]. These enzymes can degrade H_2O_2, antibiotics and other compounds, which are known to cause oxidative stress on bacteria. Different species of bacteria have different sets of catalase genes, which are activated in presence of H_2O_2. For example, *P. aeruginosa* and *E. coli* mount a strong anti-H_2O_2 response by activation of SOS signaling pathways. In a study performed by Elkin et al. activation of catalase genes *katA* and *katB* protected bacteria against lethal effects of H_2O_2 in *P. aeruginosa* biofilms [45]. Biofilms formed by a catalase mutant isolate of *P. aeruginosa* were sensitive to H_2O_2. In recent work, it was demonstrated that *P. aeruginosa* PA14 *ΔkatAB* had lower minimum biofilm inhibitory and minimum biofilm bactericidal concentrations compared to its wild type parent isolate *P. aeruginosa* PA14 [46]. Moreover, in the current work, an increase in biofilm reduction of *P. aeruginosa* PA14 *ΔkatAB* compared to its wild type parent isolate *P. aeruginosa* PA14 was observed when exposed to an H_2O_2-producing e-bandage for 24 h (Supplementary Fig. S2). Through continuous production of H_2O_2 and based on results herein and previously described [20,46], it may be possible that the described e-bandage system can overwhelm some of these oxidative stress response systems.

Results of this study demonstrate that the H_2O_2-producing e-bandage system described in this work reduces viable cell counts of mono- and dual-species biofilms *in vitro*. Future work will include testing the *in vivo* anti-biofilm activity and safety of the described e-bandage system in a mouse wound infection model.

Author contribution

Yash S. Raval: designed and performed the e-bandage experiments in the agar biofilm model, and wrote the initial manuscript draft. Abdelrhman Mohamed: designed and built wearable potentiostat, e-bandage and contributed to the experimental design. Laure Flurin: contributed to the experimental design. Jayawant N. Mandrekar: performed the statistical formal analysis on the experimental data. Kerryl E. Greenwood Quaintance: contributed to the experimental design. Haluk Beyenal: contributed to the experimental design, supervised the research project. Robin Patel: contributed to the experimental design, supervised the research project.

Declaration of competing interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioflm.2021.100055.

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