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

Yash S. Raval a, Abdelrhman Mohamed b, Laure Flurin a, Jayawant N. Mandrekar c, Kerryl E. Greenwood Quaintance a, Haluk Beyenal b, Robin Patel a,d,∗

a Division of Clinical Microbiology, Mayo Clinic, Rochester, MN, USA
b The Gene and Linda Voiland School of Chemical Engineering and Bioengineering, Washington State University, Pullman, WA, USA
c Department of Quantitative Health Sciences, Mayo Clinic, Rochester, MN, USA
d Division of Infectious Diseases, Mayo Clinic, Rochester, MN, USA

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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 (H2O2) 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 H2O2 when polarized at −0.6 VAg/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 log10 CFU/cm2 for mono- and dual-species biofilms, respectively. Results suggest that the described H2O2-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 (H2O2), 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...
particular interest in H$_2$O$_2$ and HOCl, natural biocides found in wound beds, produced as part of the cellular inflammatory response in wounds, albeit in low concentrations. H$_2$O$_2$ can improve wound healing [14,15]. H$_2$O$_2$ 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$_2$O$_2$ (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$_2$O$_2$ (or HOCl) - generating e-scaffolds was shown against bacterial and fungal mono-species and tri-species bacterial biofilms [30,31]. 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$_2$O$_2$-generating e-scaffold was transformed to a H$_2$O$_2$-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$_2$O$_2$-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 enhance 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, was designed and developed to deliver controlled amounts of H$_2$O$_2$- (and HOCl-) generating e-scaffolds was shown against bacterial and fungal mono-species and tri-species bacterial biofilms [30,31]. The e-bandage is comprised of carbon fabric, two carbon-fabric electrodes and a reference electrode, was designed and developed to deliver controlled amounts of H$_2$O$_2$ (or HOCl). Anti-biofilm activity of H$_2$O$_2$- (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$_2$O$_2$-generating e-scaffold was transformed to a H$_2$O$_2$-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$_2$O$_2$-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 enhance 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].

### 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 IDR-L6169 | Periportesisth 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 IDR-L4284 | Clinical isolate; resistant to methicillin | 2.5 μl of 0.5 McFarland growth tube |
| Staphylococcus epidermidis ATCC 35984 | Catheter septis isolate; resistant to methicillin | 2.5 μl of 3.0 McFarland growth tube |
| S. epidermidis IDR-L6461 | Periportesisth 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 IDR-L8618 | Periportesisth hip infection isolate | 2.5 μl of 1.0 McFarland growth tube |
| E. faecalis IDR-L7107 | Periportesisth knee infection isolate | 2.5 μl of 1.0 McFarland growth tube |
| E. faecalis IDR-L12374 | Periportesisth hip isolate, resistant to vancomycin and levofloxacin | 2.5 μl of 1.0 McFarland growth tube |
| E. faecium IDR-L11790 | Abscess isolate; resistant to vancomycin and penicillin, and susceptible to linezolid | 2.5 μl of 0.5 McFarland growth tube |
| Enterococci faecalis IDR-L10366 | Msap;positive isolate; resistant to cefoxafoxane/ tazobactam, imipenem, meropenem, ertapenem, ceftriaxone and cefepime | 2.5 μl of 0.5 McFarland growth tube |
| E. coli IDR-L7029 | Periportesth hip infection isolate | 2.5 μl of 0.5 McFarland growth tube |
| E. coli IDR-L6199 | Periportesth knee infection isolate | 2.5 μl of 0.5 McFarland growth tube |
| E. coli IDR-L8110 | Blood isolate | 2.5 μl of 0.5 McFarland growth tube |
| Pseudomonas aeruginosa IDR-L7262 | Periportesth hip infection isolate | 2.5 μl of 10$^8$ CFU/ml growth tube |
| P. aeruginosa Derived from ATCC 19660; (Xen 5) | Blood isolate | 2.5 μl of 10$^8$ CFU/ml growth tube |
| P. aeruginosa PAO1, ATCC 47085 | Wound isolate; type strain | 2.5 μl of 10$^8$ CFU/ml growth tube |
| P. aeruginosa PA14 | Wild type lab strain | 2.5 μl of 10$^8$ 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 ΔkatAB           | katA and katB double-knockout of PA14 | 2.5 μl of 10^5 CFU/ml growth tube         |
| *P. aeruginosa* | IDRL-11442           | Groin isolate; resistant to piperacillin/tazobactam, ceftazidime, meropenem, aztreonam, ciprofloxacin and levofloxacin and susceptible to colistin | 2.5 μl of 10^5 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, ceftizoxime, ciprofloxacin, imipenem and meropenem | 2.5 μl of 0.5 McFarland growth tube       |
| *A. baumannii* | ARLG-1268            | Wound isolate; resistant to amikacin, ampicillin, ceftriaxone and cephaloridine | 2.5 μl of 0.5 McFarland growth tube       |
| Klebsiella pneumoniae | IDRL-10377        | bla24-positive isolate; resistant to cefuroxime/tazobactam, imipenem, meropenem, ertapenem, ceftriaxone and cephaloridine | 2.5 μl of 0.5 McFarland growth tube       |
| *Bacteroides fragilis* | IDRL-11882       | Peri-prosthetic knee infection isolate | 2.5 μl of 2.0 McFarland growth tube       |
| *Citrobacter acnes* | IDRL-7676          | Peri-prosthetic 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        | Peri-prosthetic 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       |

The above table provides details on the bacterial isolates used for the study, including their respective designations, characteristics, and starting inoculum concentrations for mono-species biofilm studies.

**Biofilm quantification after e-bandage treatment:** After treatment, both Tegaderm™ and e-bands were removed from membrane biofilms. e-bands were placed in sterile Petri dishes containing 5 ml of 1 × PBS. Surfaces of the e-bands 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% CO2 atmosphere for 48 h; results were reported as CFU/cm². 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 log10 CFU/cm² and that of broth culture 0.71 log10 CFU/cm².

**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/cm². 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 H2O2-producing e-bands 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 H2O2-producing e-bands was 2.35 ± 0.92 log10 CFU/cm² (p < 0.05). The mean reduction of mono-species biofilms after 24 h exposure to H2O2-producing e-bands was 5.13 ± 1.45 log10 CFU/cm² (p < 0.05). 48 h e-bandage treatment resulted in an average reduction of 8.17 ± 0.40 log10 CFU/cm² (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 \(8.09 \pm 0.44 \log_{10} \text{CFU/cm}^2\) while that of Gram-negative mono-species biofilms was \(8.14 \pm 1.27 \log_{10} \text{CFU/cm}^2\). An outlier in the reduction trend was found with \(C.\) acnes, with earlier biofilm reductions. The average reduction of \(C.\) acnes biofilms was \(4.37 \pm 0.69 \log_{10} \text{CFU/cm}^2\) after 12 h of treatment (\(p < 0.05\)), and \(8.16 \pm 0.20 \log_{10} \text{CFU/cm}^2\) 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 \(\Delta katAB\) (an isolate that lacks \(katA\) and \(katB\) catalase genes). The average biofilm reduction for this isolate after 24 h of e-bandage treatment was \(6.59 \pm 0.11 \log_{10} \text{CFU/cm}^2\), more than the average biofilm reduction of its wild type parent isolate \(P.\) aeruginosa PA14 (\(3.52 \pm 0.12 \log_{10} \text{CFU/cm}^2\); 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 \(2.57 \pm 0.49 \log_{10} \text{CFU/cm}^2\). Mean reductions of \(4.10 \pm 0.46 \log_{10} \text{CFU/cm}^2\) 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 \(7.99 \pm 0.32 \log_{10} \text{CFU/cm}^2\) 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 \(7.11 \log_{10} \pm 1.07 \log_{10} \text{CFU/cm}^2\) whereas it was \(4.51 \pm 0.29 \log_{10} \text{CFU/cm}^2\) 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 \(7.43 \pm 1.53 \log_{10} \text{CFU/cm}^2\) when grown alone versus \(3.89 \pm 0.44 \log_{10} \text{CFU/cm}^2\) 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.

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$_2$O$_2$-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$_2$O$_2$-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$_2$O$_2$ 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$_2$O$_2$, 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$_2$O$_2$. For example, P. aeruginosa and E. coli mount a strong anti-H$_2$O$_2$ 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$_2$O$_2$ in P. aeruginosa biofilms [45]. Biofilms formed by a catalase mutant isolate of P. aeruginosa were sensitive to H$_2$O$_2$. 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$_2$O$_2$-producing e-bandage for 24 h (Supplementary Fig. S2). Through continuous production of H$_2$O$_2$ 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$_2$O$_2$-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.bioflim.2021.100055.

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