Activity of a hypochlorous acid-producing electrochemical-bandage as assessed with a porcine explant biofilm model

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Running title: Hypochlorous acid-producing e-bandage

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Equations for calculation of normalized cell viability

Normalized cell viability (NCV) was calculated by comparing viabilities before and after treatment. Equation 1 was used to calculate the NCV for each pig ear. Multiple ears were collected for biological replicates. To track viability, each pig ear was given a pig number (P#). Fluorescence of the PrestoBlue assay was activated at 535 nm and recorded at 590 nm. For each condition (no e-bandage, non-polarized e-bandage, or polarized e-bandage), viability of an explant after treatment was normalized by initial viability of the explant immediately after processing of the same pig ear.

$$P\#\ Normalized\ Cell\ Viability_{condition} = (\boldsymbol{P\#NCV_{condition}}) = \frac{Fluorescence_{590} of\ explant\ (\boldsymbol{P\#}, condition)}{Fluorescence_{590}\ of\ initial\ explant\ \boldsymbol{P\#}\ (t = 0\ days)} * 100 \quad \text{Equation 1}$$

NCVs of explants exposed to e-bandages were compared with those of explants exposed to non-polarized and polarized e-bandages. Using the NCV from the PrestoBlue assay and Equation 1, reduction in cell viability was determined. Reduction in cell viability resulting from a polarized e-bandage was calculated using Equation 2.

$$Cell\ viability\ reduced\ by\ polarized\ e-bandage = \boldsymbol{P\#NCV_{non-polarized} - P\#NCV_{polarized}} \quad \text{Equation 2}$$

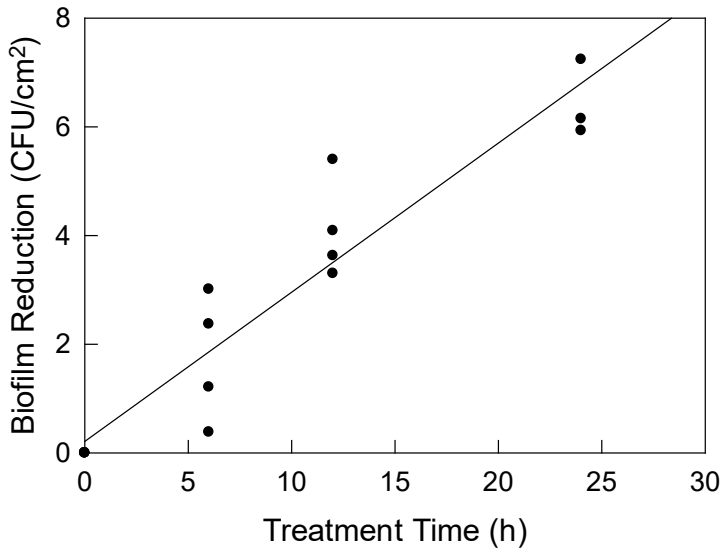

Figure S1. Log reduction (CFU/cm^2) of *Staphylococcus aureus* (IDRL-6169) biofilms with 0, 6, 12, and 24 hour (h) polarized e-bandage treatment. Biofilm reductions were linearly related to treatment times (R^2=0.98).

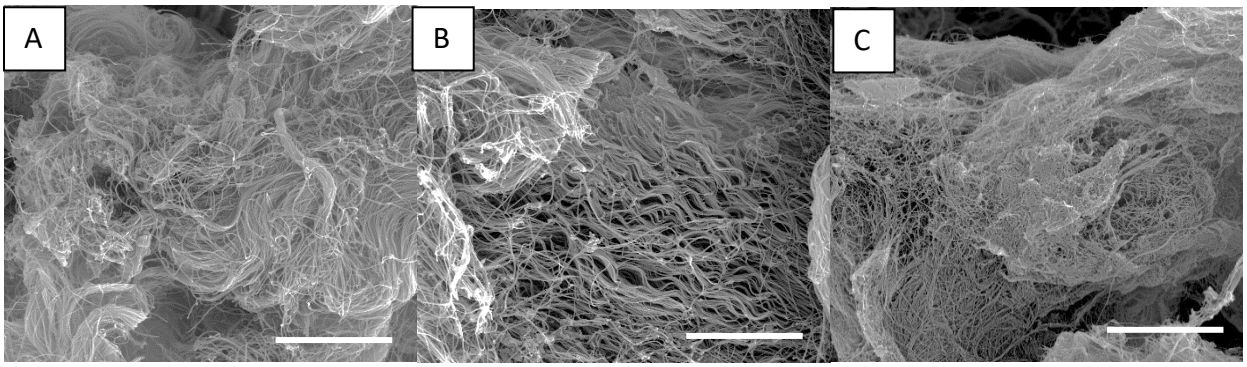

Figure S2. Uninfected explants after 12 hour exposure to e-bandages starting immediately after processing, 8000× magnification; scale bar indicates 10 µm. (A) no e-bandage, (B) non-polarized e-bandage, and (C) a polarized e-bandage. The no e-bandage- and non-polarized e-bandage-exposed explants have defined fibrous structures; the surface of the explant exposed to a polarized e-bandage is partially covered with a crust. Image contrast was modified to improve visibility.

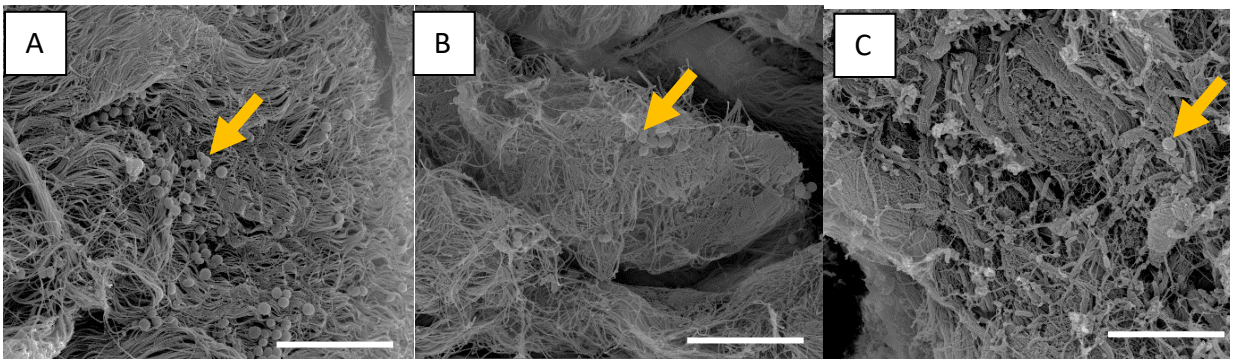

Figure S3. Infected explants after 12 hour exposure to e-bandages starting immediately after processing and infection (infection prevention model), 8000× magnification; scale bar indicates 10 µm. (A) no e-bandage, (B) non-polarized, and (C) a polarized e-bandage. The no e-bandage- and non-polarized e-bandage-exposed explants have defined fibrous structures and *Staphylococcus aureus* cells are observed on the surface of the explant between the fibers. The surface of the explant exposed to a polarized e-bandage has a crust and fewer *S. aureus* cells. Image contrast was modified to improve visibility. Arrows indicate single bacterial cell.

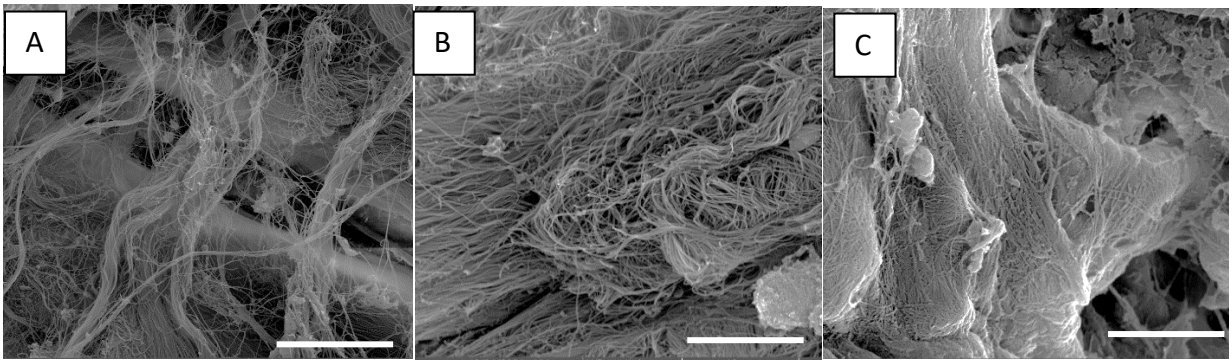

Figure S4. Three-day-old uninfected explants after 6 hour treatment exposure, 8000× magnification; scale bar indicates 10 µm. (A) no e-bandage, (B) non-polarized e-bandage, and (C) polarized e-bandage. For the no e-bandage and non-polarized e-bandage-exposed explants, the fibrous tissue is well defined. The fibers of the polarized e-bandage-exposed explant are packed together and less defined. Image contrast was modified to improve visibility.

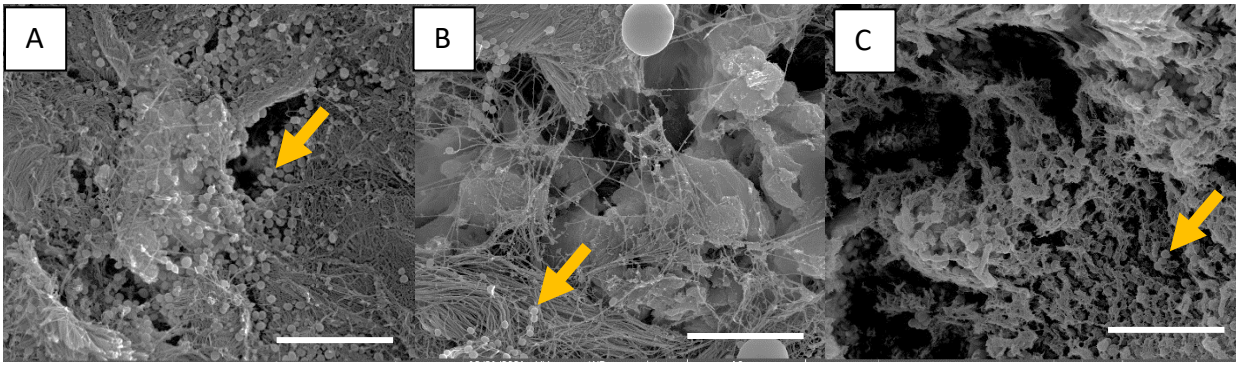

Figure S5. Three day-old *Staphylococcus aureus* IDRL-6169 infected explants (mature biofilm) after 6 hour treatment exposure, 8000× magnification; scale bar indicates 10 µm. (A) no e-bandage, (B) non-polarized e-bandage, and (C) polarized e-bandage. *S. aureus* cells are observed on and between the fibers of the explant of the no e-bandage and non-polarized e-bandage-exposed explants, and the fibers are well defined. Fewer *S. aureus* cells are observed on the surface of the explant exposed to a polarized e-bandage; a crust-like structure covers the surface. Image contrast was modified to improve visibility. Arrows indicate single bacterial cells.

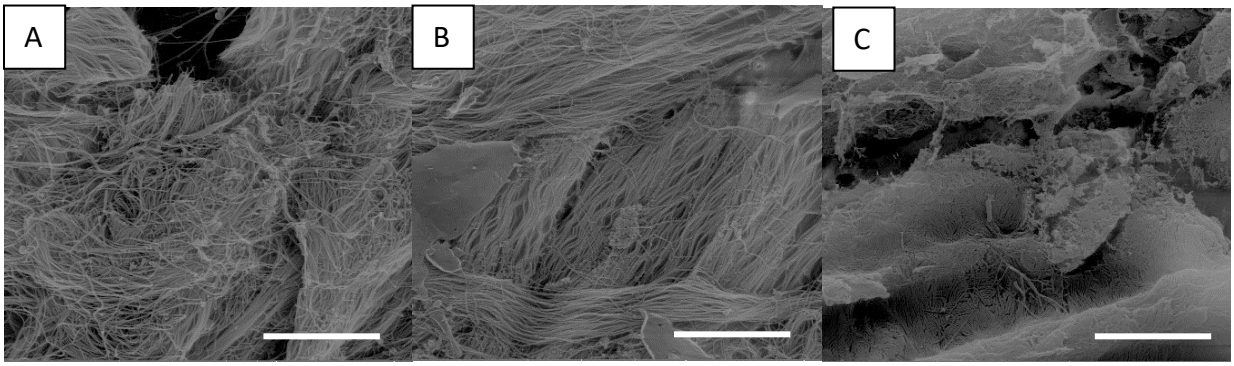

Figure S6. Three day-old uninfected explants after 12 hour treatment exposures, 8000× magnification; scale bar indicates 10 µm. (A) no e-bandage, (B) non-polarized e-bandage, and (C) polarized e-bandage. For the no e-bandage- and non-polarized e-bandage-exposed explants, fibrous tissue is well defined. Fibers of the polarized e-bandage-exposed explant are less defined, and a crust covers the surface. Image contrast was modified to improve visibility.

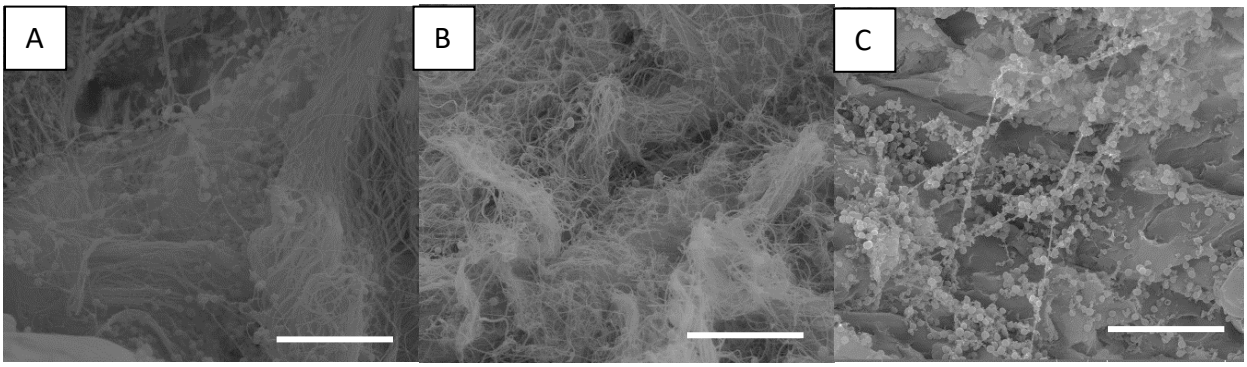

Figure S7. Three day-old *Staphylococcus aureus* (IDRL-6169) infected explants (mature biofilm) after 12 hour treatment exposures, 8000× magnification; scale bar indicates 10 µm. (A) no e-bandage, (B) non-polarized e-bandage, and (C) polarized e-bandage. For both no e-bandage and non-polarized e-bandage explants, fibrous tissue is well defined and *S. aureus* cells are observed on explant surfaces. The fibers of the polarized e-bandage-exposed explant are packed together and less defined. Strands between the *S. aureus* cells are observed more so with the polarized e-bandage- than the non-polarized e-bandage-exposed explant. Image contrast was modified to improve visibility.

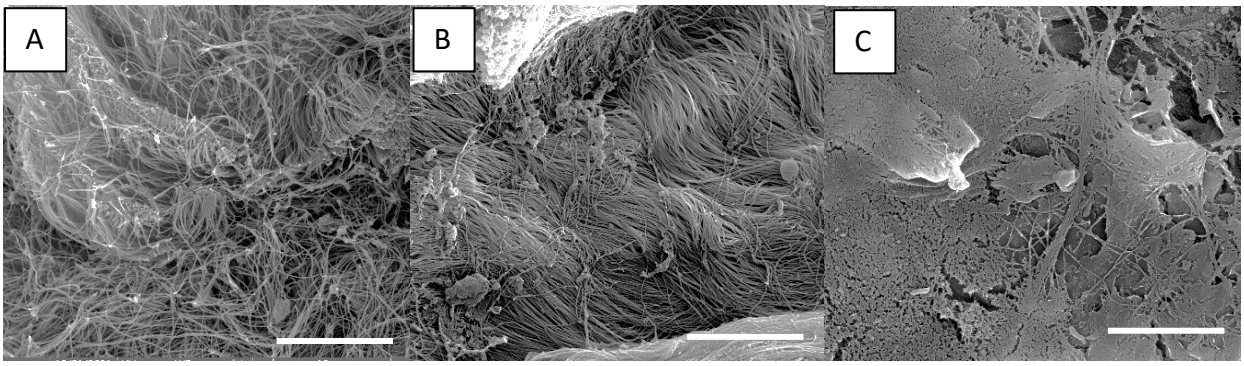

Figure S8. Three day-old uninfected explants after 24 hour treatment exposures, 8000× magnification; scale bar indicates 10 µm. (A) no e-bandage, (B) non-polarized e-bandage, and (C) polarized e-bandage. For the no e-bandage- and non-polarized e-bandage-exposed explants, fibers seen on the explant surfaces are defined and appear to flow in the same directions. A crust-like structure covers the surface of explant exposed to a polarized e-bandage. Image contrast was modified to improve visibility.

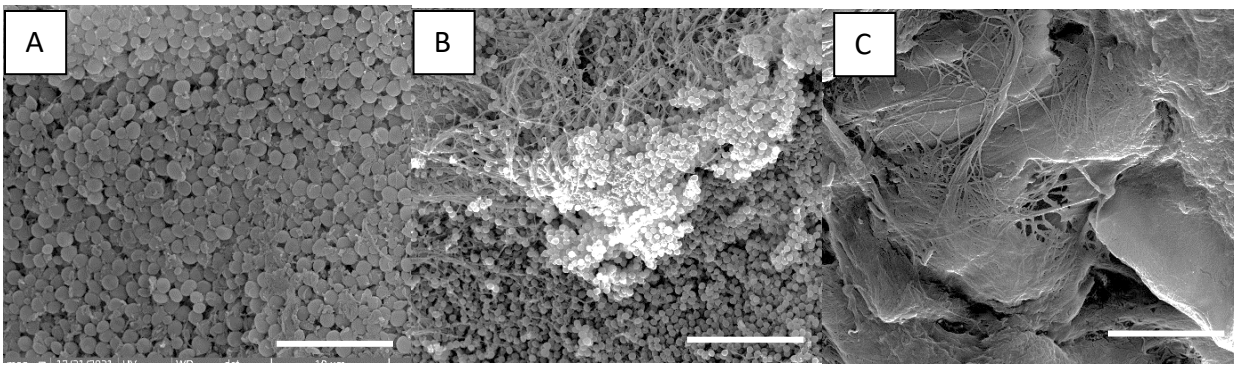

Figure S9. Three day-old *Staphylococcus aureus* (IDRL-6169) infected explants (mature biofilm) after 24 hour treatment exposures, 8000× magnification; scale bar indicates 10 µm. (A) no e-bandage, (B) non-polarized e-bandage, and (C) polarized e-bandage. The surfaces of the no e-bandage- and non-polarized e-bandage-exposed explants are covered with *S. aureus* cells whereas few *S. aureus* cells are observed on the surface of the explant exposed to a polarized e-bandage. The explant exposed to a polarized e-bandage had a crust-like cover and fewer defined fibers. Image contrast was modified to improve visibility.