

Supporting Information for  
**A highly efficacious electrical biofilm treatment system for combating chronic wound bacterial infections**

Fan Zhao\*, Yajuan Su, Junying Wang<sup>#</sup>, Svetlana Romanova, Dominick DiMaio, Jingwei Xie, Siwei Zhao\*

F. Zhao, Y. Su, J. Wang, J. Xie, S. Zhao

Mary & Dick Holland Regenerative Medicine Program, University of Nebraska Medical Center, Omaha, NE 68198, USA,

Department of Surgery, University of Nebraska Medical Center, Omaha, NE 68198 USA

<sup>#</sup>Present address: 125 Mason Farm road, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA

Email: siwei.zhao@unmc.edu, fan.zhao@unmc.edu

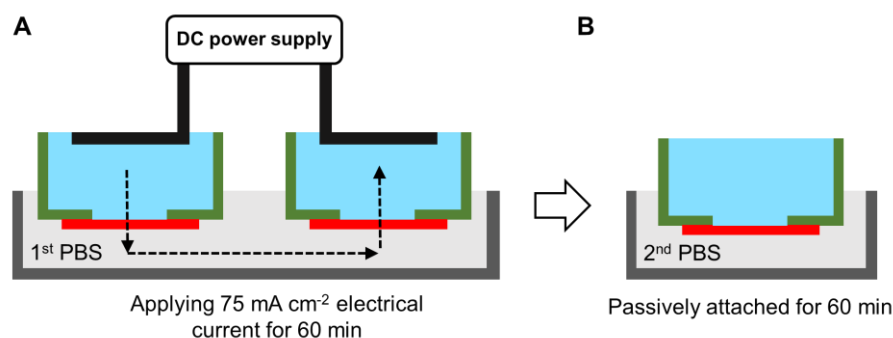
S. Romanova

Department of Pharmaceutical Sciences, University of Nebraska Medical Center, Omaha, NE 68198, USA

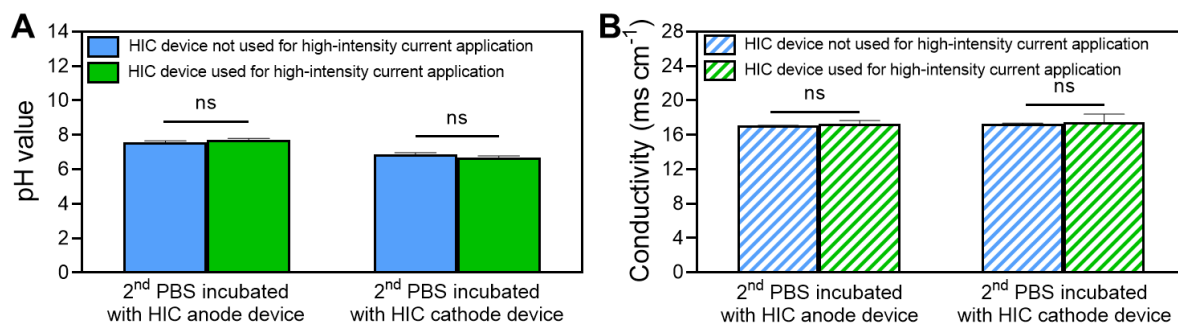
D. DiMaio

Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE 68198, USA

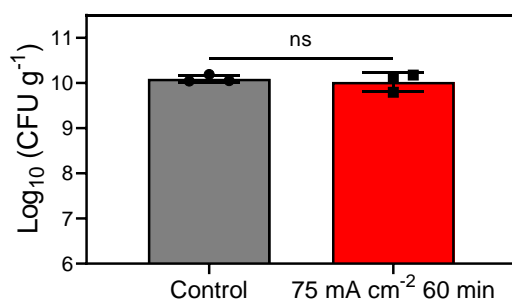
ORCID ID: 0000-0003-4436-0836 (Fan Zhao), 0000-0003-3516-9968 (Siwei Zhao)



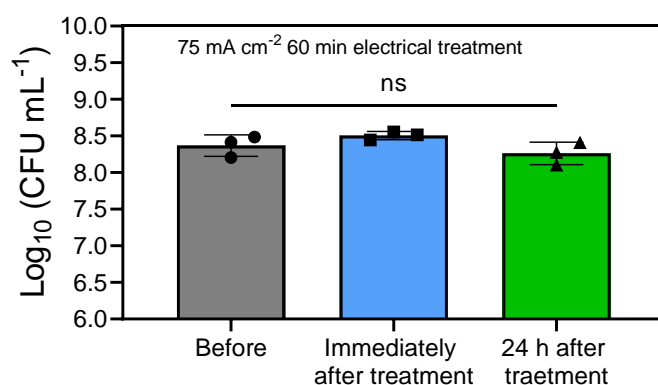
**Figure S1 Schematic illustration of PEG hydrogel stability test after high-intensity current application.** (A) Apply 75 mA cm<sup>-2</sup> current to 1<sup>st</sup> PBS for 60 minutes using HIC-based anode and cathode devices. (B) Dip HIC-based anode/cathode device in 4 mL fresh 2<sup>nd</sup> PBS for 60 minutes.



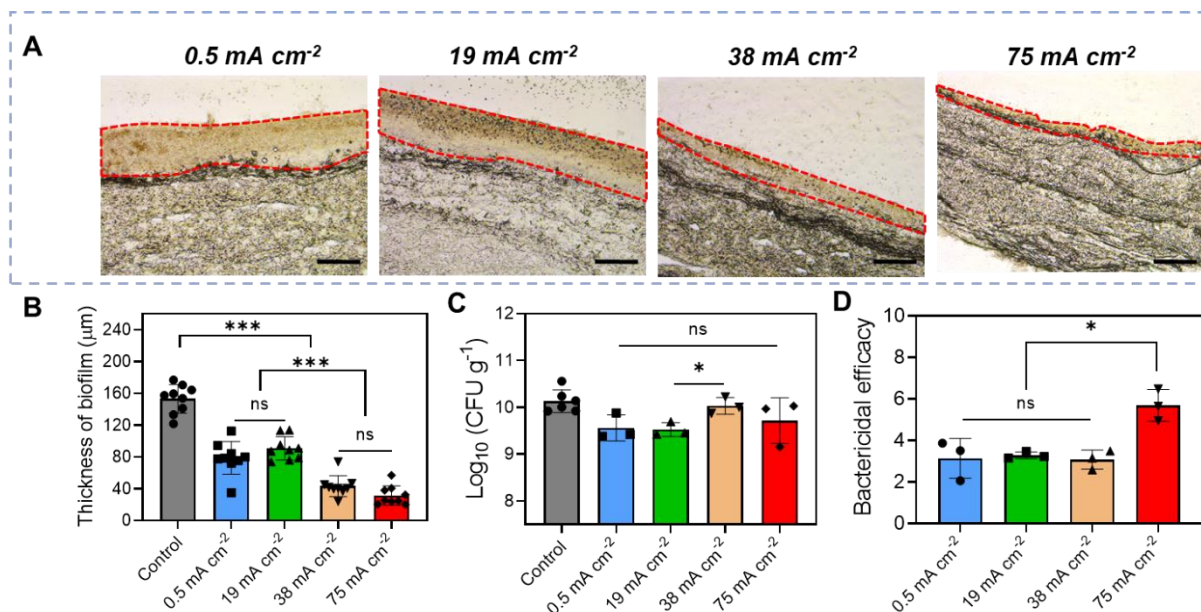
**Figure S2 Stability of PEG hydrogel in our HIC-based devices after high-intensity current application.** (A) The pH of the 2<sup>nd</sup> PBS after 60-minute incubation with HIC-based anode/cathode device that had or had not used for high-intensity current application. (B) The conductivity of the 2<sup>nd</sup> PBS after 60-minute incubation with HIC-based anode/cathode device that had or had not used for high-intensity current application. (n=3, ns for p>0.05)



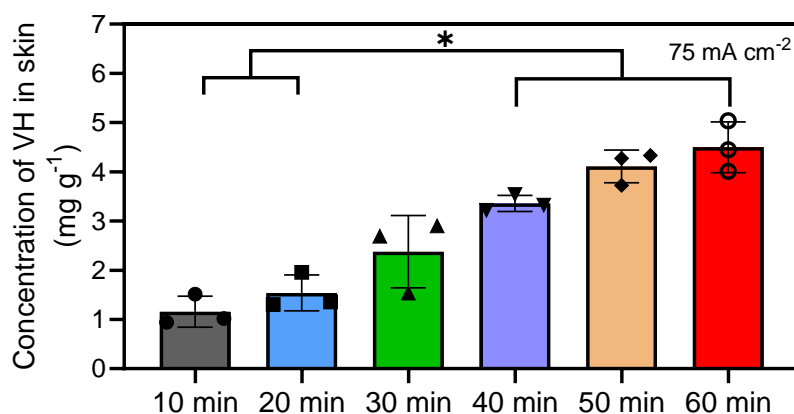
**Figure S3 Viable bacterial counts in collection solution of our working device immediately after electrical debridement (75 mA cm<sup>-2</sup> 60 min) *ex vivo*.** The result was normalized by the weight of wound tissues (per gram) and compared with the bacterial count of the untreated biofilm.



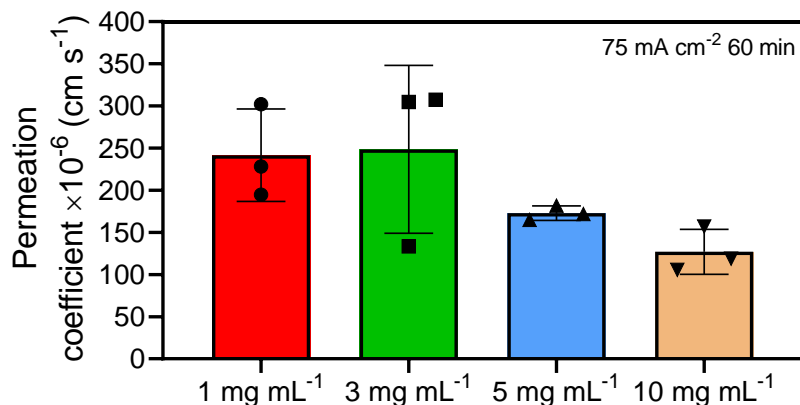
**Figure S4 Viable planktonic MRSA bacteria counts before and after 75 mA cm<sup>-2</sup> 60 min treatment *in vitro* conducted by our HIC-based system.**



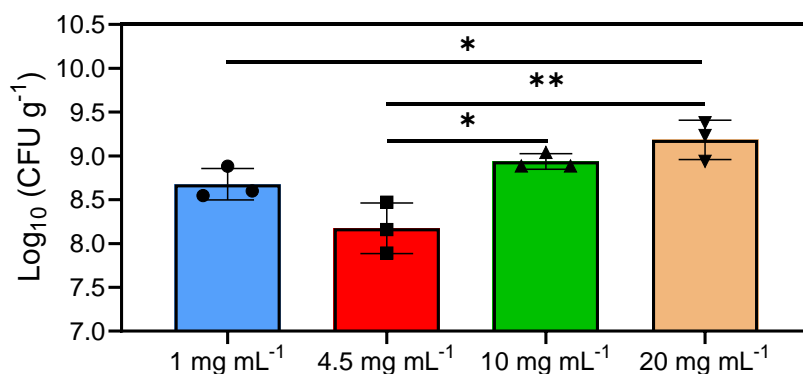
**Figure S5 Restoration of biofilm at 24 h after electrical debridement.** (A) Representative cryo-section images of *ex vivo* biofilm infected skin wound samples, which were incubated in 37 °C incubator for 24 h after electrical debridement. Scale bar: 100  $\mu\text{m}$ . (B) Biofilm thickness at 24 h after electrical debridement. (C) Biofilm bioburden at 24 h after electrical debridement characterized by the standard plate counting assay. (D) Bactericidal efficacy at 24 h after electrical debridement.



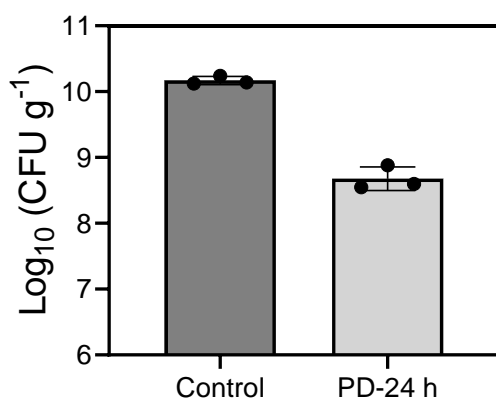
**Figure S6 Accumulated concentrations of VAN in biofilm-skin wound samples as a function of electrical treatment time** (1  $\text{mg mL}^{-1}$  VAN was loaded in drug chamber and applied 75  $\text{mA cm}^{-2}$  current by using HIC-based system).



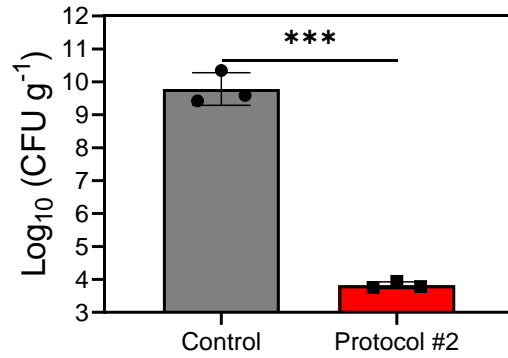
**Figure S7** Permeability coefficient of VAN after 75 mA cm<sup>-2</sup> 1-h iontophoresis using HIC-based system loaded with different concentrations of VAN.



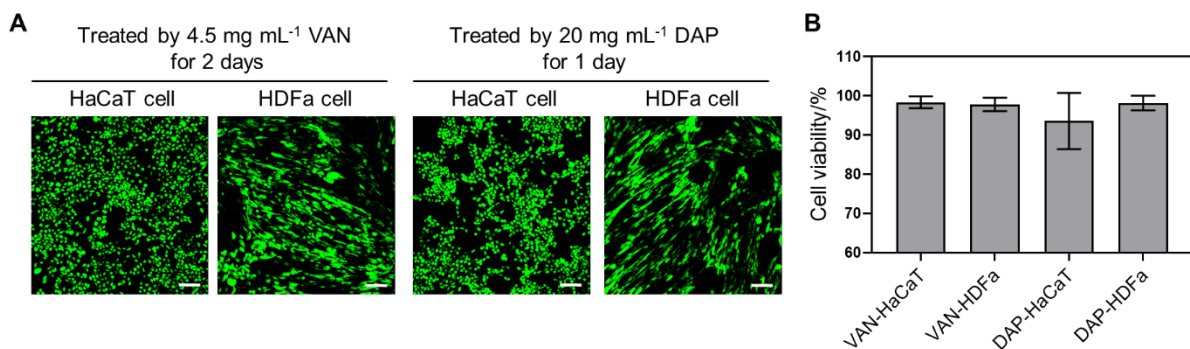
**Figure S8** Bacteria counts after 1 mg mL<sup>-1</sup>, 4.5 mg mL<sup>-1</sup>, 10 mg mL<sup>-1</sup>, and 20 mg mL<sup>-1</sup> VAN topically treated *ex vivo* mature MRSA biofilm for 24 h (passive diffusion).



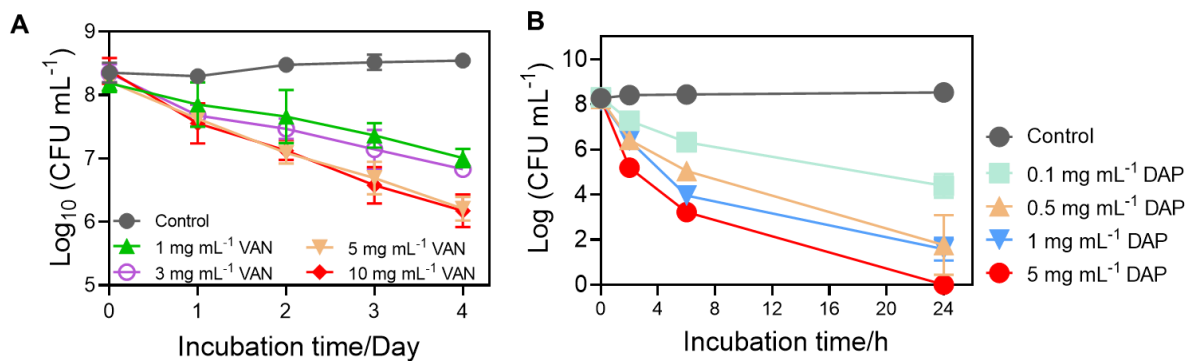
**Figure S9** Bactericidal results after 1 mg mL<sup>-1</sup> VAN passive diffusion for 24 h (PD-24h) on the *ex vivo* biofilm infected skin wound.



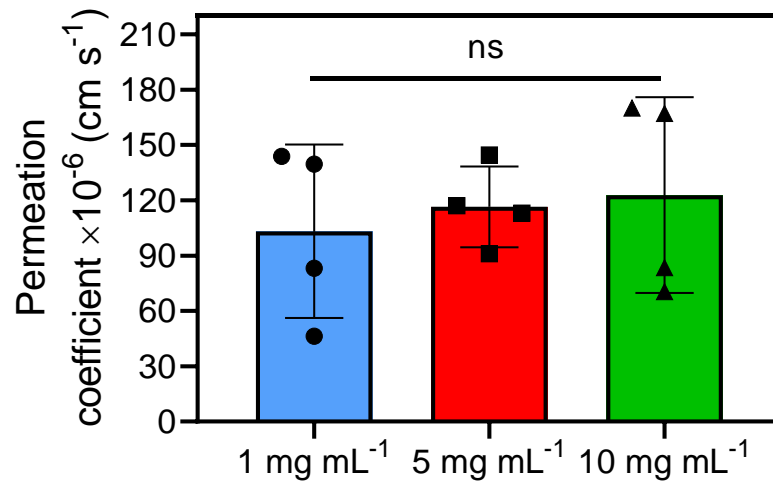
**Figure S10** Bacteria counts of *ex vivo* skin wound biofilm at 4 days after protocol #2 treatment.



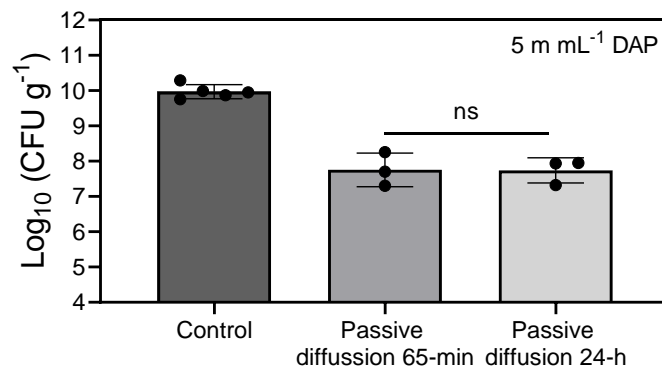
**Figure S11** LIVE/DEAD images and cell viabilities of HaCat cells and HDFa cells after treated by high concentration antibiotics *in vitro*. (A) LIVE/DEAD images; (B) Cell viability calculated from LIVE/DEAD images in ImageJ software. VAN-HaCaT: HaCaT cell treated by 4.5 mg mL<sup>-1</sup> vancomycin for 2 days; VAN-HDFa: HDFa cells treated by 4.5 mg mL<sup>-1</sup> vancomycin for 2 days; DAP-HaCaT: HaCaT cell treated by 20 mg mL<sup>-1</sup> daptomycin for 1 day; DAP-HDFa: HDFa cell treated by 20 mg mL<sup>-1</sup> daptomycin for 1 day.



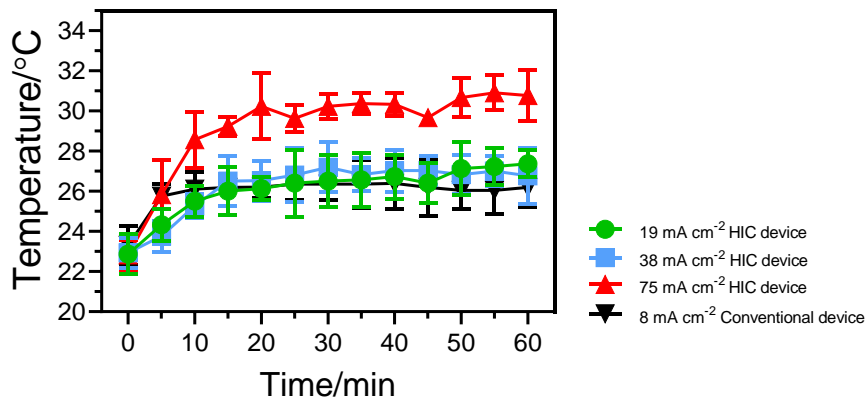
**Figure S12** Evaluation of VAN activity as a function of time. (A) Comparative time-killing kinetics of MRSA cells in stationary phase (4-day time period) after VAN treatment by standard plate counting assay. (B) Comparative time-killing kinetics of MRSA cells in stationary phase (4-day time period) after DAP treatment by standard plate counting assay.



**Figure S13** Permeation coefficient of DAP iontophoresis ( $75 \text{ mA cm}^{-2}$ ) in biofilm infected skin wound *ex vivo* using our biofilm treatment system with different loading concentrations in drug chamber.

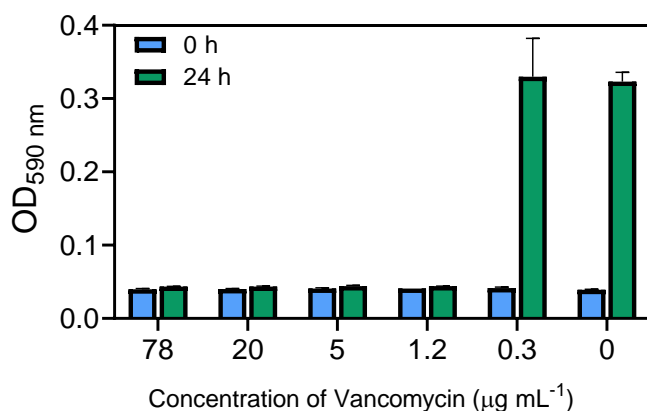


**Figure S14** Bactericidal efficacy of DAP passive diffusion ( $5 \text{ mg mL}^{-1}$ ) for 65-min and 24-h, respectively, on the *ex vivo* biofilm infected wounds.

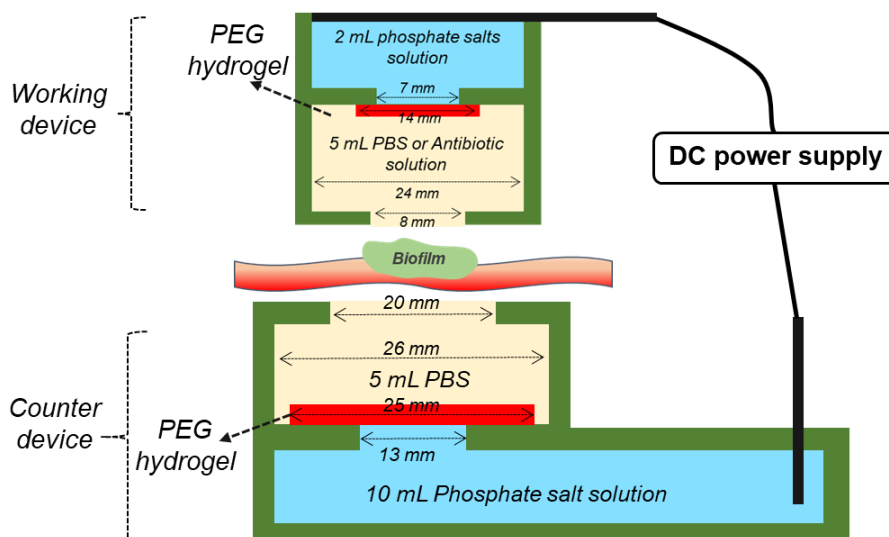


**Figure S15** *In vivo* temperature-versus-time curve on the skin surface during different

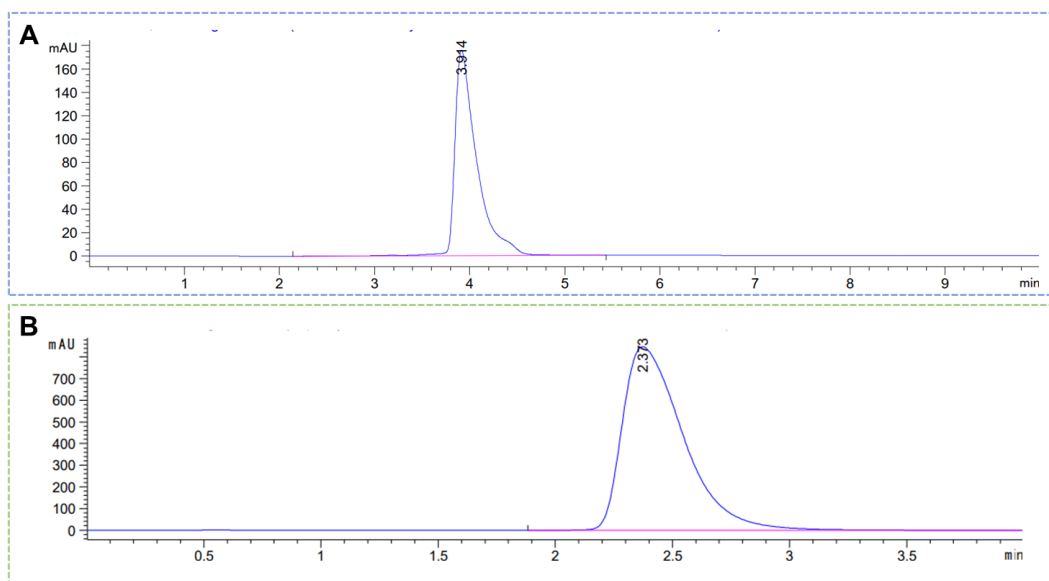
current intensity applications at  $19 \text{ mA cm}^{-2}$ ,  $38 \text{ mA cm}^{-2}$ , and  $75 \text{ mA cm}^{-2}$  for 60 min using HIC-based system and  $8 \text{ mA cm}^{-2}$  60 min application using conventional device, respectively



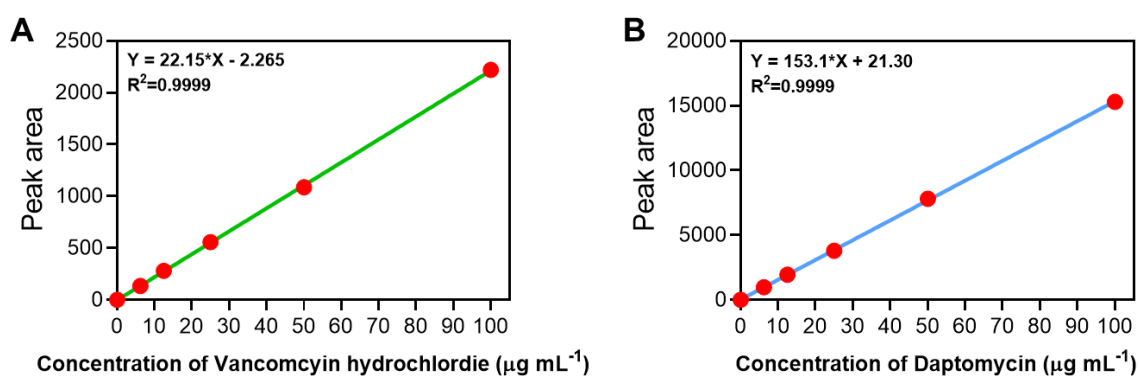
**Figure S16 Minimal inhibitory concentration (MIC) of VAN to planktonic MRSA.**



**Figure S17 Schematic test setup and dimension of our biofilm treatment system for *ex vivo* electrical debridement, drug delivery and biofilm treatment studies.** The counter device shown here had a slightly different design than the working device to allow easy and secure placement of the skin/wound tissue and easy insertion of the carbon electrode.



**Figure S18 Chromatogram showing a 100 mg L<sup>-1</sup> antibiotic standard solution sample (A) Vancomycin hydrochloride, (B) Daptomycin**



**Figure S19 Standard calibration curve of (A) Vancomycin hydrochloride and (B) Daptomycin concentrations ranging from 6.25 μg mL<sup>-1</sup> to 100 μg mL<sup>-1</sup>, respectively, using optimized HPLC methods**