Transient Biofilm Susceptibility Induced by Electric Fields Enables Near-Complete Mature Staphylococcal Eradication

Marco Balato and Carlo Petrarca

Department of Electrical Engineering and Information Technologies (DIETI), University of Naples "Federico II", Napoli (NA), Italy

Biofilms, defined as complex communities of microorganisms encased within a self-produced extracellular matrix, have been identified as a major contributor to chronic and device-associated infections. Their highly organized structure and altered metabolic states confer extraordinary tolerance to antimicrobial agents, often resulting in persistent infections that are recalcitrant to conventional antibiotic therapies. Methicillin-resistant Staphylococcus aureus (MRSA) biofilms pose a severe clinical challenge, leading to prolonged hospitalization, increased healthcare costs, and elevated morbidity and mortality rates. Despite the advances in antimicrobial development, the eradication of established biofilms remains a challenge, particularly because antibiotic penetration is impeded by the dense Extracellular Polymeric Substance (EPS) matrix, and sessile bacteria within biofilms are in dormant states that render them less susceptible to antibiotics targeting active cellular processes. Consequently, there is a need for adjunctive strategies that can transiently disrupt biofilm integrity and enhance the efficacy of conventional antimicrobial treatments. Physical methods, including the application of electric fields, have emerged as a promising adjunct for the control of biofilms. Low-intensity electric fields have been shown to influence bacterial membrane potentials, increase permeability, and enhance the susceptibility of biofilm-associated bacteria to antibiotics [1-2]. The "bioelectric effect", which was initially described as the enhancement of antibiotic action, has opened new avenues for the combination of physical and chemical treatments against biofilms [3-4]. However, the activity and temporal dynamics of this phenomenon remain poorly understood. The present study aims to address this knowledge gap by investigating whether electric field exposure induces a transient window of vulnerability during which biofilm-embedded bacteria become susceptible to antibiotics. Furthermore, the critical role of treatment timing – whether antibiotics should be applied concurrently with or after electrical exposure – has not been systematically addressed. The present study hypothesize that low-intensity electric fields induce a temporary, reversible phase of increased biofilm susceptibility, which is herein defined as the "Electric Field–Induced Vulnerability Window." Utilizing mature 96-h mature *S. aureus* biofilms as a model, this study evaluates the impact of synchronous and asynchronous antibiotic application relative to electrical stimulation. The findings of this study demonstrate that synchronous treatment results in significant biofilm eradication, whereas delayed antibiotic administration does not fully exploit the induced vulnerability. The results obtained establish a new conceptual framework for timing-optimized biofilm therapies and suggest novel strategies for overcoming the resilience of pathogenic biofilms through the synergistic use of physical and chemical agents.

In vitro experimental results

An integrated platform was developed for the application of low-intensity electric fields to mature biofilms and their electrical characterization. The impact of low- intensity electric field exposure (rms electric field amplitude equal to 12.5 mV/cm), either as a standalone treatment or in combination with vancomycin, on 96-h mature *S. aureus* biofilms was evaluated through colony-forming unit (CFU) enumeration, as outlined in Figure 1. The application of the electric field, either in isolation or in conjunction with vancomycin for a duration of four minutes, did not yield a statistically significant reduction in viable cell counts when compared to untreated controls ($P > 0.05$) [5]. Specifically, electric field exposure alone for a duration of four minutes resulted in a negligible reduction in CFUs, and the administration of vancomycin in both considered modes did not yield any additional effect ($5 \times 10^8 \pm 1.53$ vs. $8 \times 10^8 \pm 1$ CFU/mL, $P = 0.06$). Conversely, extending the electric field exposure to six minutes led to a substantial reduction of CFUs, achieving at least an 83.3% decrease compared to 5-day-old untreated biofilms ($5 \times 10^7 \pm 1$ vs. $3 \times 10^8 \pm 1.52$ CFU/mL, $P=0.03$).

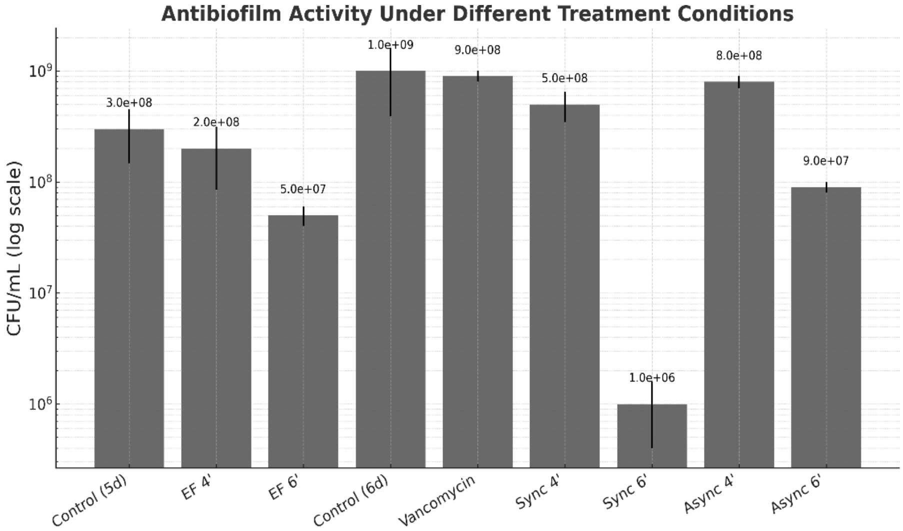

Figure 1: Reduction in colony-forming units (CFU/mL) across different treatment conditions applied to mature Staphylococcus aureus biofilms. Data are expressed as mean ± standard deviation. Treatments included Electric Field (EF) exposure alone for 4 or 6 minutes, vancomycin application, and combined electric field plus antibiotic treatments applied either synchronously or asynchronously. A logarithmic scale was used to better represent the wide range of bacterial loads.

References

[1] Del Pozo, J. L., & Patel, R. (2009). The challenge of treating biofilm-associated bacterial infections. Clinical Pharmacology & Therapeutics, 86(2), 203–209. https://doi.org/10.1038/clpt.2009.122.

[2] Giladi, M., Porat, Y., Blatt, A., Wasserman, Y., Wasserman, E., Lubart, R., & Lavie, R. (2008). Microbial killing by low- intensity electric fields. Antimicrobial Agents and Chemotherapy, 52(10), 3517–3522. https://doi.org/10.1128/AAC.00222-08.

[3] Blake, R. C., & Revis, N. W. (1991). Electrochemical enhancement of biocidal activity against Pseudomonas aeruginosa biofilms. Applied and Environmental Microbiology, 57(7), 2120–2125. https://doi.org/10.1128/aem.57.7.2120-2125.1991.

[4] Costerton, J. W., Ellis, B., Lam, K., Johnson, F., & Khoury, A. E. (1994). Mechanism of electrical enhancement of efficacy of antibiotics in killing biofilm bacteria. Journal of Industrial Microbiology, 13(3), 232–236. https://doi.org/10.1007/BF01584001.

[5] Balato, M., Vitelli, M., Petrarca, C *et al.* On the effectiveness of electrical characterization of mature Staphylococcal Biofilm. *Sci Rep* **15**, 8445 (2025). https://doi.org/10.1038/s41598-025-93300-1