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Evaluating the Efficacy of Dielectric Barrier Discharge Plasma against Planktonic and Biofilm Cultures of *Staphyloccocus aureus*

Irena Maliszewska, Anna Zdubek
Wrocław University of Science and Technology, Wybrzeże Wyspiańskiego 27, Wrocław, Poland

INTRODUCTION & AIM

Staphylococcus aureus is a highly adaptable pathogen recognized for its capacity to create biofilms, which are organized clusters of bacteria surrounded by a protective extracellular matrix. The formation of biofilms increases its ability to cause disease by shielding it from the host's immune system and enhancing its resistance to antibiotics [1]. Biofilms are often linked to chronic infections, particularly those associated with medical devices and implants, where *S. aureus* can survive and avoid elimination. Effective methods for disrupting biofilms include the use of antimicrobial agents, physical removal techniques, and advanced technologies such as ultrasound or electrochemical treatments. Understanding how biofilms form and resist treatment is essential for creating effective strategies to prevent and manage infections and contamination related to biofilms.

Plasma is recognized as the fourth state of matter, following solid, liquid, and gas, and is composed of a blend of excited molecules, charged particles, reactive oxygen species (ROS), reactive nitrogen species (RNS), and ultraviolet (UV) light [2]. Recently, nonthermal plasma has attracted significant interest as a safe technology. This type of plasma is produced under low-pressure or atmospheric conditions and primarily utilizes energy in the form of electrons, rather than heating the entire gas system. This method is effective for disinfecting and sterilizing various materials, particularly those that may be adversely affected by heat treatment. Nonthermal plasma is thought to generate various toxic agents with strong bactericidal properties, with ROS and RNS being the primary biocidal factors. Several mechanisms of plasma's bactericidal action have been identified, which include the oxidation and perforation of cell membranes, degradation and alteration of proteins, modification and disruption of nucleic acid chains, as well as interference with extracellular polymeric substances (EPS) and quorum sensing (QS) in biofilms.

The aim of our research was to investigate the biocidal effect of non-thermal plasma on suspended cells and biofilms of *Staphylococcus aureus*, as well as to evaluate the impact of multiple exposures of bacteria to plasma on their biofilm formation capacity.

METHOD

The bacterial strain *Staphylococcus aureus* used in the study was sourced from the Polish Collection of Microorganisms (PCM 2560). A sample of an overnight culture was centrifuged at 500 g for 5 minutes, and the supernatant was discarded. The resulting pellet was re-suspended in sterile phosphate buffered saline to create an inoculum of roughly 1.0×10^8 colony-forming units ($CFUmL^{-1}$). Glass plates measuring 10 mm x 10 mm and 2 mm thick were sterilized in an autoclave at 121 °C for 20 minutes. Subsequently, 20 µl of the standardized bacterial suspension was placed onto the surface of the glass plate, resulting in a contamination level of 2.0×10^7 CFU/plate. The plate was then incubated at 37 ºC for 24 hours. After this time, the medium was discarded and the biofilm was washed with sterile buffered saline to remove planktonic cells. The suspension culture was eliminated by placing the bacteria on a glass slide, allowing them to dry gently, and then exposing them to plasma.

The plasma inactivation of bacteria on the glass plates was conducted using a DBD reactor operating at atmospheric pressure with air as the working gas. All glass plates were placed in the reactor, but only the inoculated side was exposed to the DBD plasma for durations ranging from 1 to 10 minutes. After exposure, the plates were transferred to sterile tubes containing sterile buffered phosphate saline and gently vortexed. The concentration of surviving cells was assessed using the serial dilution method.

Multiple plasma treatments. Glass plates inoculated with a bacterial suspension/biofilm were subjected to DBD plasma treatment to achieve a 90% pathogen mortality rate (sublethal dose). Following plasma exposure, the plates were placed in 5 ml of Mueller-Hinton II broth and incubated for 24 hours at 37 °C. The cells were then collected through centrifugation and resuspended in buffered saline to create an inoculum of approximately 1.0×10^8 colony-forming units (CFU/mL). This suspension was used to inoculate additional plates which were also treated with a sublethal dose of plasma. This process was repeated 10 times. The bacterial culture that did not undergo plasma treatment was labeled as B0, while cultures subjected to ten and fifteen exposures to sublethal doses of plasma were labeled as P10. respectively.

The bacteriological killing efficiency was also determined by the MTT assay. Reduce the yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to purple formazan crystals by metabolically active cells as an indicator of their viability was carried out according to the procedure described by Grela et al. [3]. The metabolic activity of the cells was expressed in [%], where 100% was the absorbance value of formazan (A_{540}), which was formed after incubation of the tetrazolium salt with biofilm prior to plasma treatment.

RESULTS DISCUSSION

The mortality rate of planktonic bacteria was found to be contingent upon the duration of dielectric barrier discharge (DBD) plasma exposure, with a 5-minute treatment resulting in a 4.0 ± 0.05 $\log_{10}$ reduction in viable bacterial cells. A complete lethality, characterized by a reduction in viable cell counts below the detection threshold, was observed after 6 minutes of DBD plasma exposure. Assessment of cellular redox potential utilizing the MTT assay corroborated that plasma treatment induced a significant level of cell death, with metabolic activity of the bacterial population diminishing by 85±3% relative to the control group, wherein 15±3% of cells in the planktonic suspension remained viable following 6 minutes of plasma exposure. Furthermore, it was estimated that a DBD plasma treatment duration of 2 minutes and 22 seconds resulted in a 90% reduction in viable cell counts (equivalent to a 1.0 $\log_{10}$ decrease), categorizing this energy dose as sublethal. As anticipated, the efficacy of biofilm removal was inferior compared to that of planktonic cells, with cell mortality reaching approximately 89 ± 0.5% after 10 minutes of plasma treatment (Fig. 1).

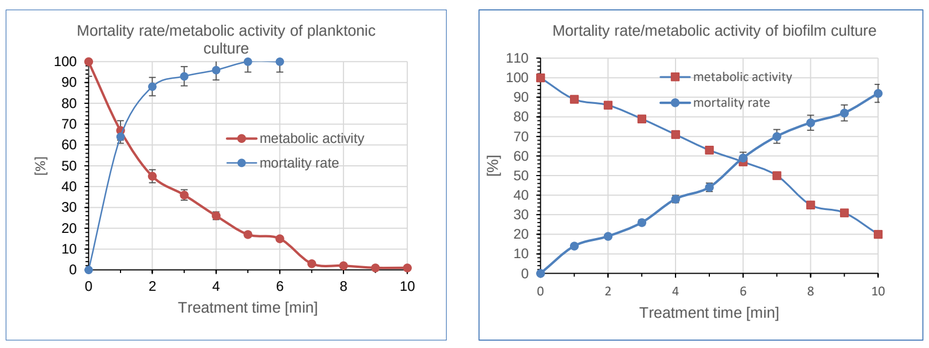

Figure 1. The effect of DBD plasma on the mortality rate and bacterial redox potential of planktonic and biofilm formed *by S. aureus*. Average ±SD of three independent experiments is shown.

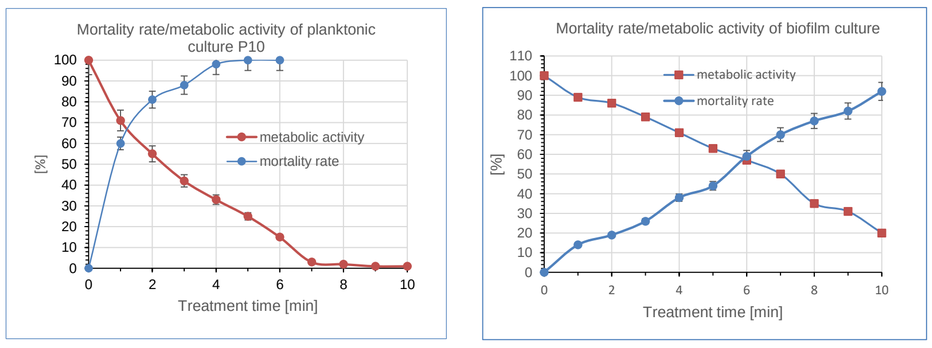

Figure 2. The effect of DBD plasma on the mortality rate and bacterial redox potential of planktonic and biofilm formed *by S. aureus P10*. Average ±SD of three independent experiments is shown.

The findings indicate that the effectiveness of biofilm eradication for the bacteria subjected to plasma treatment ten times (P10) was notably high and varied with the duration of plasma exposure (Figure 2). The most significant biofilm removal efficiency was recorded after 10 minutes of plasma treatment, resulting in a reduction of bacterial counts by 92%. An analysis of the redox potential of the bacteria (P10) within the biofilm revealed that their metabolic activity after 10 minutes of plasma exposure was only 20% of the initial activity (prior to plasma treatment).

CONCLUSION

This research emphasizes the notable effects of dielectric barrier discharge (DBD) plasma on both planktonic and biofilm cultures of *S. aureus*, revealing varying levels of susceptibility to treatment. Planktonic bacteria exhibited high mortality rates with relatively brief exposure to DBD plasma, whereas biofilms demonstrated greater resistance, necessitating longer treatment times to achieve comparable cell death rates. Despite the difficulties presented by biofilms, our findings indicate that multiple exposure to sublethal doses of DBD plasma does not result in resistance development in *S. aureus* cells.

FUTURE WORK / REFERENCES

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