ANTIMICROBIAL EFFICACY OF COLD ATMOSPHERIC PRESSURE PLASMA JET AGAINST CLINICAL BACTERIAL ISOLATES

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Abstract

The alarming rise of antimicrobial resistance (AMR) necessitates the development of novel antimicrobial strategies. In this aspect, cold atmospheric pressure plasma jet (CAPJ) has sparked research interest for its novel antimicrobial properties and has found its applicability in sterilization, disinfection, and decontamination. The CAPJ discharges are composed of reactive species such as reactive oxygen and nitrogen species (RONS), electrons, ions, and excited atoms and molecules, which incorporate an antimicrobial nature. Although CAPJ has proven highly efficient in bacterial load reduction, certain unresolved problems must be addressed systematically to establish CAPJ as a prominent antimicrobial technique. Firstly, CAPJ's efficacy against clinically isolated multidrug-resistant (MDR) bacteria has not been adequately studied. Secondly, to achieve optimal productivity, it is imperative to systematically characterize the developed CAPJ device based on operational parameters concerning antimicrobial activity against MDR isolates. Thirdly, it is important to assess the time-dependent bacterial inactivation process that CAPJ reactive species follow. Fourthly, there has been limited studies on CAPJ's efficacy in eliminating bacterial load from inanimate surfaces, along with any alteration in surface property. Lastly, there is a dearth of research comparing the antimicrobial activity of various CAPJ activated liquids (CAPJALs) and comprehending the specific parameters required for enhanced efficacy. The goals of the current study were to address the aforementioned problems.

In the thesis, CAPJ powered by a low-frequency AC source was designed and developed for antimicrobial applications. A thorough investigation of the CAPJ's operational parameters (intrinsic and extrinsic) and their influence on discharge characteristics, reactive species composition, and antimicrobial activity was carried out. Various reactive species such as excited atoms (Ar I, He I, O I, etc.), ions (Ar^+, N_2^+, N^+, etc.), radical RONS (OH•), and non-radical RONS (OH^+, NO^+, $N_2O_3^-$, NO_3^-, etc.) were observed to vary in the discharge depending on the intrinsic settings (voltage, frequency, gas flow rate, and operating gas). This variance in reactive species in relation to intrinsic settings was associated with varying bacterial inactivation area. Extrinsic parameters such as exposure distance, exposure time, bacterial concentration, and type of target bacteria also influenced the antimicrobial activity of CAPJ.

The study also examined the induced probable bacterial inactivation path by Ar CAPJ. A greater than 6 $\log_{10}$ reduction of *E. coli* and *S. aureus* within 60 and 120 s CAPJ exposure was noticed, respectively, along with low *D*- values. The correlation between the inactivation curve

and the time-dependent damage to bacterial cells revealed varying inactivation processes among isolates. The alteration of Fourier transform infrared spectra (FTIR) and Raman micro-spectra signals of post-CAPJ exposed bacteria demonstrated the degree of destruction at the molecular level, such as lipid peroxidation, protein oxidation, bond breakages, etc. Further, the transmission electron microscopy (TEM) images of exposed bacteria indicated incurred damages on cell morphology by CAPJ reactive species.

A greater than 5 $\log_{10}$ reduction of *E. coli* and ~ 3.4 – 4.6 $\log_{10}$ reduction of *S. aureus* along with *D*- value in the range 27 – 63 s was observed over the material test surfaces on Ar CAPJ exposure. In addition, the study examined the effect of repeated CAPJ exposure on surface property by replicating hospital surface decontamination. A non-linear variation in the surface properties, such as wettability, roughness, and elemental composition was noticed. Specifically, the CAPJ reactive species would play a significant role in bacterial load reduction and any surface property alteration occurring.

The Ar CAPJ was used to activate liquids such as deionized water (DI-W), drinking water (DW), tap water (TW), and normal saline (NS), and its antimicrobial property was evaluated against *E. coli* and *S. aureus*. The computed *D*- value followed the trend – DI-W ≈ NS > DW > TW. An optimal setting for liquid activation by CAPJ and CAPJAL – bacterial interaction time was noticed to achieve higher bactericidal efficacy. Further, the pace at which the physicochemical parameters (electrical conductivity (EC), total dissolved solids (TDS), pH, and reactive species concentration (H_2O_2, NO_3^-, and NO_2^-)) changed within the liquid differed. The interaction of CAPJ reactive species with the liquid would lead to the generation of liquid phase reactive species (NO_3^-, NO_2^-, H^+, H_2O_2, etc.), contributing to physicochemical properties variation and incorporation of antimicrobial nature.

An in-depth grasp of controlling CAPJ parameters involving antimicrobial activity and the emergence of unique pathways for bacterial inactivation could be advanced by this research. The study on CAPJ's efficiency in decontaminating inanimate surfaces and CAPJALs insights CAPJ to be used as a point-of-care antimicrobial technique. The findings of this thesis would be a great asset to multidisciplinary researchers.