Computational Investigation of Mechanisms Leading to Planktonic Cell Death using Touching and Non-touching Atmospheric Pressure Plasma Jets

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Abstract: A reaction mechanism for cold atmospheric (CAP) treatment of idealized planktonic cells in solution was developed for use in a 0-dimensional, plug-flow plasma chemistry model. Cell kill curves were predicted for two CAP configurations – the kINPen, in which plasma "touches" the liquid and the COST-jet in which reactive species diffuse from the plasma to the liquid. A comparison of time to cell death is presented. Differences in mechanisms leading to cell killing in both configurations are discussed.

Keywords: plasma-liquid interactions, plasma modelling, plasma medicine, atmospheric pressure plasma

1. Introduction

Cold atmospheric plasmas (CAPs) are of interest to the plasma medicine community as non-invasive sources of reactive oxygen species (ROS). Two CAP configurations, the kINPen and the COST-jet, have been widely studied as ROS sources for applications in wound healing, cancer therapy, disinfection, and sterilization [1-3]. However, mechanisms for interactions of plasma-produced ROS with living organisms, in particular interactions that result in reduction of cell viability, are still unclear. In general, the introduction of plasma-produced ROS to a bacterial or cancer cell containing medium is thought to induce apoptosis through oxidative stress. ROS interactions at the cell membrane result in modifications that enable ROS to enter the cell, resulting in reduced functionality of vital cell processes [4].

Studies addressing the mechanisms of bacterial cell inactivation using the kINPen and the COST-jet have been performed independently, however, there are few direct comparisons of the two devices. Lackmann et. al [5] investigated how modifications of the biological molecule cysteine differed when treated with CAP produced by both configurations. When a cysteine solution was treated for 10 minutes with the same Ar/O_2 gas mixture, the kINPen produced more severe modifications than the COST-jet. Mechanisms derived from plasma-produced ROS interactions with organics in solution can be used as a basis for understanding CAP effects on cell death.

In this work, we computationally investigated the consequences of ROS generation by the CAP devices based on the kINPen, capable of directly treating a solution, and COST-jet, limited to remote treatment, operating in Ar/O_2 mixtures on the treatment of idealized planktonic cells (colony forming units, or CFUs) in a water solution. A reaction mechanism based on ROS interactions with simple organic molecules in solution was extended to describe interactions with CFUs that likely lead to cell death. Differences in the time required to produce a 2-log reduction in cell viability using the CAP configurations are discussed. Mechanisms that result in ROS delivery to the liquid are also discussed.

2. Description of the Model

GlobalKin is a 0-dimensional plasma chemistry model capable of addressing gas flow, circuitry, plasma-surface, and plasma-liquid interactions. *GlobalKin* has been described in detail elsewhere and so will be described only briefly here [6]. The electron temperature is given by solution to the electron-energy equation. Species continuity equations that account for sources and losses due to reactions and transport are solved to provide species densities. *GlobalKin* provides the option to approximate gas-flow using a plug-flow approach in which the gas travels in the flow direction as a slug having the reactor cross-sectional area. The plug speed is a function of inlet flow rate, total gas density, gas heating and the reactor cross-sectional area.

GlobalKin is capable of addressing plasma-liquid interactions across a boundary layer [6,7]. All gas-phase species are given a liquid-phase counterpart. Neutral gas-phase species diffuse across the gas-liquid boundary and solvate into the liquid with a with a probability determined by Henry's law equilibrium. Liquid-phase reactions are included as a separate mechanism. Fluences (cm^{-2}) of gas-phase ROS to the liquid are calculated using gas-phase produced fluxes.

Cell death is approximated within the current reaction mechanism framework as a series of sequential reactions with ROS that progressively damage the cell. Based on a typical bacterium size and surface site density, our idealized planktonic cell is assumed to have 10^8 reaction sites. The cell is considered no longer viable when 10% of the sites have undergone a reaction with ROS in solution, which would be 10^7 discrete events. Reaction rate coefficients for reactions of cells (colony forming units or CFUs) are approximated based on rates of hydrogen abstraction from the simple biological molecule cysteine [8]. The mechanism and rate coefficients for one step of sequential cell death are shown in Table 1.

Since it is not practical to track the 10^7 sequential reactions on individual cells required to declare the cell dead, the following procedure was followed. A total of 19 sequential generations of increasingly modified CFUs were tracked. The 20^{th} generation is declared dead. The rate of consumption of ROS is scaled by the ratio of actual cell sites needed to kill a cell ($0.1 \times 10^8 = 10^7$) to the number of modifications required to kill the model CFUs to reflect probable reactions rates with real cells.

Table 1. CFU Mechanism

Reaction[a]	Reaction Rate Coefficient (cm^3/s)
$CFU_n + O_{(aq)} \rightarrow CFU_{n+1}$	1.68×10^{-15}
$CFU_n + O^-_{(aq)} \rightarrow CFU_{n+1} + e_{(aq)}$	1.68×10^{-20}
$CFU_n + OH_{(aq)} \rightarrow CFU_{n+1}$	1.68×10^{-17}
$CFU_n + OH^-_{(aq)} \rightarrow CFU_{n+1} + e_{(aq)}$	1.91×10^{-20}
$CFU_n + H_2O_2 \rightarrow CFU_{n+1} + H_2O_{(aq)}$	4.68×10^{-22}
$CFU_n + HO_{2(aq)} \rightarrow CFU_{n+1}$	5.65×10^{-16}

[a] n increases sequentially until n+1 is a dead CFU. $n+1 \leq 20$ for this work. Dead cells continue to react with ROS but are not tracked for number of reacted sites.

3. A Model for CAP Produced Cell Death

ROS interactions with CFUs that result in a 2-log normal reduction in healthy CFUs were estimated for the CAPs with operating conditions patterned after the kINPen and the COST-jet.

The kINPen operates by applying a high-frequency voltage to a stainless steel pin electrode contained in a quartz tube. To enable direct side-by-side comparison with the COST-jet, the power used in our kINPen model was 1 W. while acknowledging that the kINPen usually operates with higher power. The electrode length was 1 cm. There is 0.2 cm gap between the end of the pin electrode and the liquid surface in which the plasma is exposed to ambient air. The total inlet gas flowrate was 1000 sccm. In the kINPen configuration for this work, the plasma touches the liquid surface.

The COST-jet consists of two electrodes, one grounded and one powered at radio frequency, contained in a grounded housing. The electrode length is 3 cm and the electrode width is 0.1 cm. The gap between the two electrodes is 0.1 cm. The plasma effluent exits the reactor and mixes with ambient air in the gap between the outlet and the liquid surface. The plasma does not touch the liquid. For our COST-jet model, the power was also 1 W and the air gap was 0.2 cm. The total gas inlet flowrate was 1000 sccm.

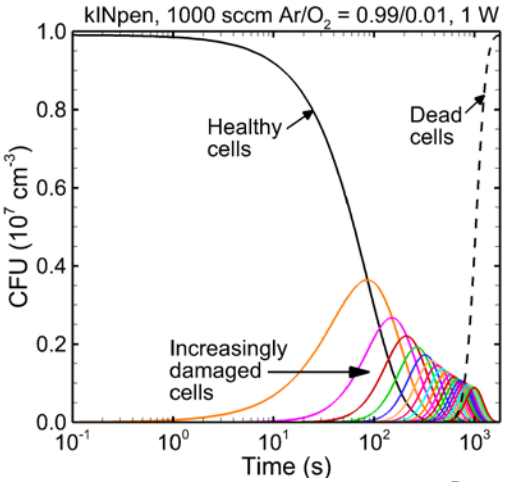

Fig. 1. Cell death for initial density of 10^7 cm^{-3} CFUs for 30 minutes treatment with Ar/O_2 kINPen.

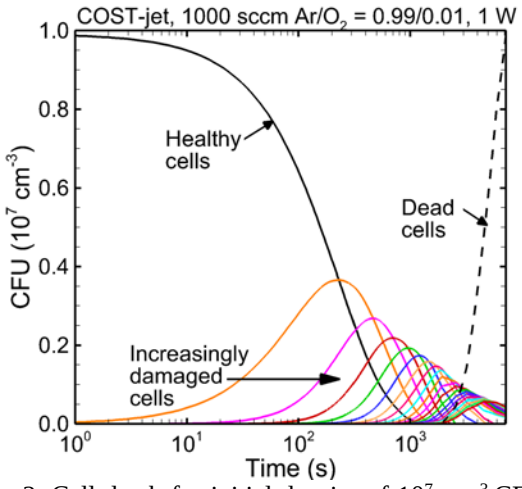

Fig. 2. Cell death for initial density of 10^7 cm^{-3} CFUs after 2 hours of treatment with Ar/O_2 COST-jet.

To compare the effects of ROS delivery to CFUs for both base case conditions, the same gas composition of Ar/O_2 = 99%/0.1% was used. A small water impurity (10 ppm) was included to emulate experimental conditions. Cell death curves for the base case kINPen and COST-jet are shown in Fig. 1 and Fig. 2, respectively.

For Ar/O_2 kINPen treatment of a water solution with an initial CFU density of 10^7 cm^{-3}, the model predicts 2-log reduction in healthy CFUs after 5 minutes of treatment, while an additional 5-6 minutes of treatment is required to produce noticeable densities of dead cells. (A healthy cell has not reacted with ROS.) All of the cells are dead after 30 minutes of plasma treatment. This result is consistent with timescales required for bacterial inactivation by the kINPen reported in the literature [9].

To produce the same 2-log reduction in healthy cells with the same gas composition in the Ar/O_2 COST-jet, longer treatment times were required. After about 20 minutes of treatment by the COST-jet, healthy cells experience nearly a 2-log reduction. However an additional 15 minutes of treatment (35 minutes total) is required to begin to see significant production of dead cells. After 2 hours of plasma treatment, about 98% of the cells are dead.

When the power, inlet flow rate, air gap distance, and inlet gas composition are the same in both reactors, the gas-phase production of the ROS responsible for cell death is higher in the COST-jet (5.5×10^{15} cm^{-3} ROS in the center of the reactor) than the kINPen (3.9×10^{15} cm^{-3} ROS at the midpoint of the kINPen. The difference in ROS production can be attributed to the differences in reactor geometries that result in different power deposition profiles.

Though more gas-phase ROS are produced by the COST-jet, less gas-phase ROS make it through the 0.2 cm air gap to the liquid surface. For example, the density of gas-phase O at the liquid surface is 2.3×10^{15} cm^{-3} in the COST-jet case and 3.6×10^{15} cm^{-3} in the kINPen case. There are also considerably more negatively charged ROS species that make it to the liquid surface in the kINPen (1.9×10^9 cm^{-3} O^-, 8.9×10^6 cm^{-3} OH^-) than in the COST-jet,

where the gas-phase densities of negatively charged ROS species at the liquid surface are so small as to be negligible.

The larger quantities of ROS that make it to the surface of the liquid when using the kINPen can be attributed to the "touching" nature of the kINPen in this configuration. In this kINPen configuration, the density of electrons at the surface of the liquid is 5.2×10^9 cm^{-3}. As the plasma is not shielded from the ambient air, more ROS can be generated in the air gap above the liquid surface by electron impact reactions. With the COST-jet being "nontouching," electrons are depleted by attachment to ROS in the air gap with their density being reduced to negligible values at the liquid surface. Reactions in the air gap are dominantly between heavy species and often result in depletion of the ROS required to produce cell death (for example, the depletion of O by three-body reactions to form O_3).

In the "touching" configuration, gas-phase electrons solvate into the liquid and can drive production of ROS by aqueous reaction, which contributes to higher densities of aqueous ROS. For example, the saturated density of O_{aq} in the kINPen configuration is 7.6×10^{11} cm^{-3} and 4.9×10^{11} cm^{-3} COST-jet configuration. The ability of the kINPen to produce ROS by aqueous reactions, combined with the higher density of gas-phase ROS at the liquid surface that will saturate into the liquid, leads to higher fractional cell death when using the touching plasma. Similar fractional cell death can likely be achieved using the COST-jet by increasing ROS fluence to the liquid by, for example, changing the gas-composition, operating power, or changing the distance between the reactor outlet and the liquid surface.

4. Concluding Remarks

A reaction mechanism based on ROS interactions with organic molecules was extended to include ROS interactions with CFUs that result in cell death. The mechanism was implemented in a 0D plasma chemistry model to provide predictions for 2-log reduction in CFU viability using two different CAP sources – at touching configuration based on the kINPen and a non-touching design based on the COST-jet. Treatment by the kINPen results in a 2-log reduction in cell viability and production of dead CFUs within 10 minutes while the COST-jet requires in excess of 30 minutes. Differences in the necessary treatment times to produce dead CFUs can be attributed to the differences in aqueous ROS production mechanisms. Plasma produced by the kINPen touches the plasma (aqueous reactions produce ROS) whereas production of aqueous ROS by the COST-jet is primarily due to transport from the reactor to the liquid surface. Comparison of cell-killing by CAP sources having different mechanisms of aqueous ROS production can provide insight into the mechanisms responsible for cell death for plasma medical applications. Knowledge gained from these insights will enable more efficient design of plasma sterilization and therapeutic devices.

5. References

[1] J. W. Lackmann and J. E. Bandow, Appl. Microbiol. and Biotechnol, **98**, (2014).
[2] S. Bekeschus, et. al. Clinical Plasma Med., **4**, 1 (2016).
[3] Y. Gorbanev, et. al. Plasma, **2**, 3 (2019).
[4] J. Van der Paal, et. al. Chem. Sci., **7**, 489 (2016).
[5] J. W. Lackmann, et. al., Sci. Rep. **8**, 1 (2018).
[6] A. Lietz and M. J. Kushner. J. Phys. D. Appl. Phys., **49**, 42 (2016).
[7] J. Kruszelnicki, et. al. J. Phys. D. Appl. Phys., **52**, 35 (2019).
[8] J. Polito, M. J. Herrera Quesada, et. al., *Manuscript in preparation*, (2023).
[9] C. A. J. van Gils et. al., J. Phys. D. Appl. Phys., **46**, 175203, (2013).

6. Acknowledgments

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