Relating plasma-activated water chemistry to antimicrobial effectiveness by pairing EPR spectroscopy and single-cell IFC

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Abstract: Here, we investigate how variations in liquid-phase chemistry translate to biological outcomes of *E. coli* exposed to plasma-activated water (PAW). Unique insights are derived through the employment of two powerful diagnostics: EPR spectroscopy and single-cell IFC. The results indicate that, even in cases of indirect plasma treatment, short-lived reactive species can have a strong influence on inactivation pathways.

1. Introduction

The antimicrobial effectiveness of plasma-activated water (PAW) is well-documented and attributed to the reactive oxygen and nitrogen species (RONS) present in the liquid [1]. Recent studies from our group [2,3] have highlighted this property of PAW at two distinct time points: immediately following production ("fresh") and after 72 hours. Interestingly, both are capable of reaching the sterilization threshold for *E. coli* (log-6 reduction) despite distinct chemistries. This provides an opportunity to investigate the chemical kinetics of PAW and the corresponding biological response.

Here, two diagnostics are employed in parallel to do so. The first, electron paramagnetic resonance (EPR) spectroscopy, is used to scrutinize the liquid-phase chemistry. The second, single-cell impedance flow cytometry (IFC), provides a rapid, label-free technique to probe modifications at a subcellular level [4]. Together, these two diagnostics can offer unique insights into the link between PAW chemistry and antimicrobial effectiveness.

2. Methods

The PAW used in this study was produced with a surface dielectric-barrier discharge (sDBD) ignited in air. For both PAW storage times, a 30-minute treatment of *E. coli* achieved a log-6 reduction [2,3].

EPR measurements were performed with two different nitroxides, TEMPOL and PTIO. The degradation of the former was used to probe short-lived reactive oxygen species (ROS) [5], while the conversion of the latter to PTI indicated the presence of nitric oxide (NO).

Single-cell IFC data were collected over a frequency range of 1–20 MHz, allowing for the derivation of dielectric dispersion curves for each measured population. From this, the dielectric parameters of individual subcellular layers could be estimated.

3. Results and Discussion

Figure 1 illustrates the differences in PTI production and TEMPOL degradation between fresh and 72-hour PAW. These results indicate that fresh PAW efficiently produces NO, likely as a result of HNO_2 decay. This, and other pathways involving acidified nitrites, have been identified as the basis for the antimicrobial properties of fresh PAW. However, PAW stored for 72 hours does not contain high concentrations of nitrite, suggesting that different pathways are responsible for inactivating *E. coli*. This is further supported by the observed TEMPOL degradation. As shown in Figure 1, the data imply that pathways involving long-lived RONS may be producing potent short-lived ROS as intermediaries, leading to cell death. Additionally, changes in PAW chemistry translate to the dielectric spectra of the PAW-treated bacteria, as recorded by single-cell IFC. The measured dielectric spectra are then used to identify differences in subcellular modifications between the two populations and form a hypothesis as to the pathways leading to inactivation.

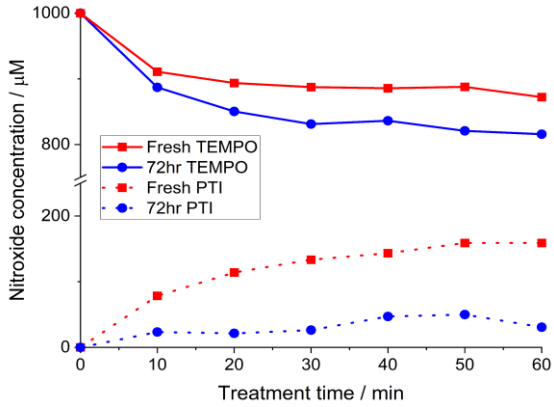

Fig. 1. The PTI production and TEMPOL degradation for fresh and 72-hour PAW.

4. Conclusion

In this study, EPR spectroscopy and single-cell IFC are used to investigate how variations in PAW chemistry and short-lived intermediaries result in distinct biological outcomes. RONS concentrations probed with EPR spectroscopy are compared with modifications of subcellular dielectric properties derived from single-cell IFC. This work provides a blueprint for advancing mechanistic understanding and tailoring device design to achieve intended biological outcomes.

References

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