## Strategies to enhance the antimicrobial activity of plasma activated water

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**Abstract:** Plasma activated water (PAW) usually comprises a mixture of hydrogen peroxide  $(H_2O_2)$ , nitrite  $(NO_2^-)$  and nitrate  $(NO_3^-)$  and has a low pH. The combination of these low molecules combined with its low pH are thought to give PAW its antibacterial qualities not readily achievable by other methods. However, certain bacteria such as *Staphylococcus aureus* and biofilms can be resistant to PAW treatments. Therefore, strategies are required to enhance the antimicrobial property of PAW to mitigate potential antimicrobial resistance. This presentation will cover strategies based on plasma source design optimisation and additional of chemical additives that we have been implementing to enhance the oxidation potential and consequently antimicrobial activity of PAW.

Keywords: Plasma activated water, antimicrobial resistance, bacteria.

Antimicrobial resistance (AMR) continues to be a major health and environmental challenge. Widespread overuse of antibiotics has escalated the problem. As such urgent non-antibiotic strategies urgently needed to mitigate AMR.

Plasma activated water (PAW) has shown to be a promising antibiotic-free strategy for controlling antibacterial growth in potential applications of medicine, agriculture, and food hygiene. PAW is typically prepared by treating water with a low-temperature atmosphericpressure plasma to generate a complex mixture of oxidants such as hydrogen peroxide ( $H_2O_2$ ), less reactive molecules such as nitrite ( $NO_2^-$ ) and nitrate ( $NO_3^-$ ) and acids that lower the solution pH. The combined action of these molecules at low pH are thought to give PAW its antibacterial properties.

Although PAW is generally effective at decontaminating different types of bacteria, in certain situations microbes have shown to be resistant to PAW. This is particularly true for bacterial biofilms which can house strong antioxidant defence mechanisms to protect resident bacteria from oxidative attack. To overcome potential AMR to PAW, and to enhance its antimicrobial effectiveness, we have been developing bespoke plasma systems and new chemical formulations to enhance the oxidative potency and consequently bactericidal activity of PAW.

In the new plasma systems, we have optimised production of  $H_2O_2$  in plasma jets by increasing the length of the dielectric tube and increasing spacing of multiple ground electrodes as shown in Fig. 1 [1, 2]. The plasma jet enhances  $H_2O_2$  production through electron-induced dissociation reactions and UV photolysis within the plasma core and in the plasma afterglow. These processes will be further explained in my talk. We have also shown that we can further enhance  $H_2O_2$  production by assembling the optimally configured plasma jet into array of plasma jets that essentially increases the volume of the reaction

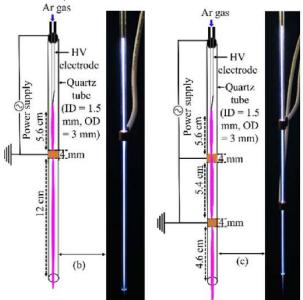


Fig. 1. Schematics and photographs of a single (left) and double (right) ground electrode plasma jet in operation. The relatively long dielectric tube and spacing between electrodes enhances production of  $H_2O_2$ .

chamber (i.e. the dielectric tubes). But the array of plasma jets need to be in a conical configuration to avoid electrostatic, hydrodynamic, and photolytic interference between each of the plasma jets to achieve reliable operation (Fig. 2) [3]. Using this configuration, we can achieve a 9-fold enhancement in the  $H_2O_2$  production rate.

In addition, we have also improved the chemistry of PAW with the addition of acetyl donor antimicrobial precursor molecules (Fig. 3) [4]. We investigated tetraacetylethylenediamine (TAED) and pentaacetate glucose (PAG) acetyl donors. We showed that plasma can be used to readily activate the TAED and PAG to generate a potent antimicrobial formulation to decontaminate common wound pathogens as well as SARS-CoV-2.

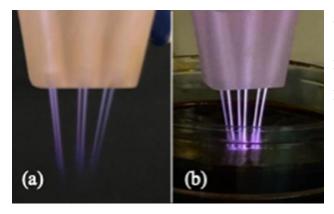


Fig. 2. Photograph of conical assembly of 6 plasma jets operated into (a) ambient air and (b) into water for preparation of PAW. The conical assembly of plasma jets increase  $H_2O_2$  production by 9-fold compared to a single plasma jet.

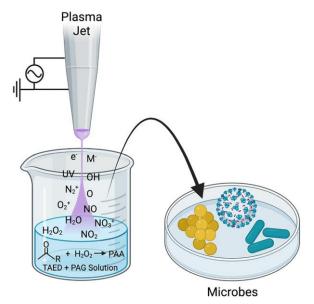


Fig. 3. Overview of the method used to plasma activate TAED and PAG acetyl donors to generate a potent antimicrobial PAW formulation.

Overall, the combined optimisation of plasma jets with acetyl donors that can further enhance the bactericidal activity of PAW, may lead to more effective antimicrobial strategies in the future. Importantly, this method is also environmentally-friendly as it negates the need of antibiotics and the by-products of PAW and acetyl donors decompose to biocompatible products.

## 1. References

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