Developing a plasma functionalised liquid for the treatment of orthopaedic implant associated infections

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Introduction

Orthopaedic implant infections are a huge burden to patients and healthcare services globally. Current treatments include wound debridement and treatment with antibiotics, however in some cases these treatments are not adequate, and the device needs to be removed surgically[1]. These infections are particularly difficult to treat as bacteria can form biofilms on the surface of the implants and the increasing prevalence of antimicrobial resistant species including *Staphylococcus aureus* and *Pseudomonas aeruginosa* species. Cold atmospheric plasma is an ionized gas which contains short- and long-lived reactive Oxygen and Nitrogen species. To date, cold atmospheric plasma treatments have shown potential for preventing and controlling these orthopaedic infections due to their wound healing properties and antimicrobial activity. Liquids exposed to cold atmospheric plasma have been shown to have similar properties. These liquids may be a possible solution in treating surgical and device associated infections.

This project aims to investigate the use of plasma functionalised liquids (PFLs) generated from several devices to treat different phenotypes of S. aureus and P. aeruginosa infections. The plasma devices under investigation include an in-house reactive species specificity (RSS) system, a submerged DBD system for variable volume and the MiniMIP system courtesy of Dr Ehlbeck, INP.

Specific Aims

- Characterise the principal reactive species in PFLs generated by different plasma devices
- Assess the efficacy of PFLs against *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains
- Optimise a PFL for treatment of biofilms
- Understand the PFL efficacy in sequential and combination therapy development

Methods

- Plasma devices

Table 1: Summary of plasma devices and generation parameters used

System	Generation time	Water Volume treated	Power	Energy	Energy/mL
RSS - Spark	30min	10mL	27W	48.6kJ	4.86 kJ/mL
RSS - Glow	30min	10mL	27W	48.6kJ	4.86 kJ/mL
Mini-MIP	30min	100mL	70W	126kJ	1.26 kJ/mL
Submerged DBD	15min	50mL	400W	720kJ	14.4 kJ/mL

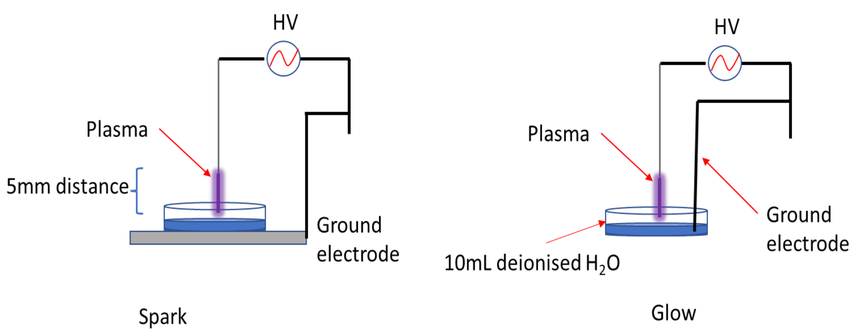

Figure 1: Diagram of RSS Spark and Glow configurations

- Suspension assays to assess efficacy against planktonic bacteria
- Colorimetric assays to assess reactive species concentration

Bacterial Strains

Table 2. List of bacterial strains under investigation

Staphylococcus aureus	*Pseudomonas aeruginosa*
Staphylococcus aureus USA 300	*Pseudomonas aeruginosa PA01 ATCC BAA-47*
MRSA ATCC 33592	*P. aeruginosa* ATCC 27853
Staphylococcus aureus ATCC 25923	

Plasma functionalised liquids antimicrobial activity against *S. aureus*

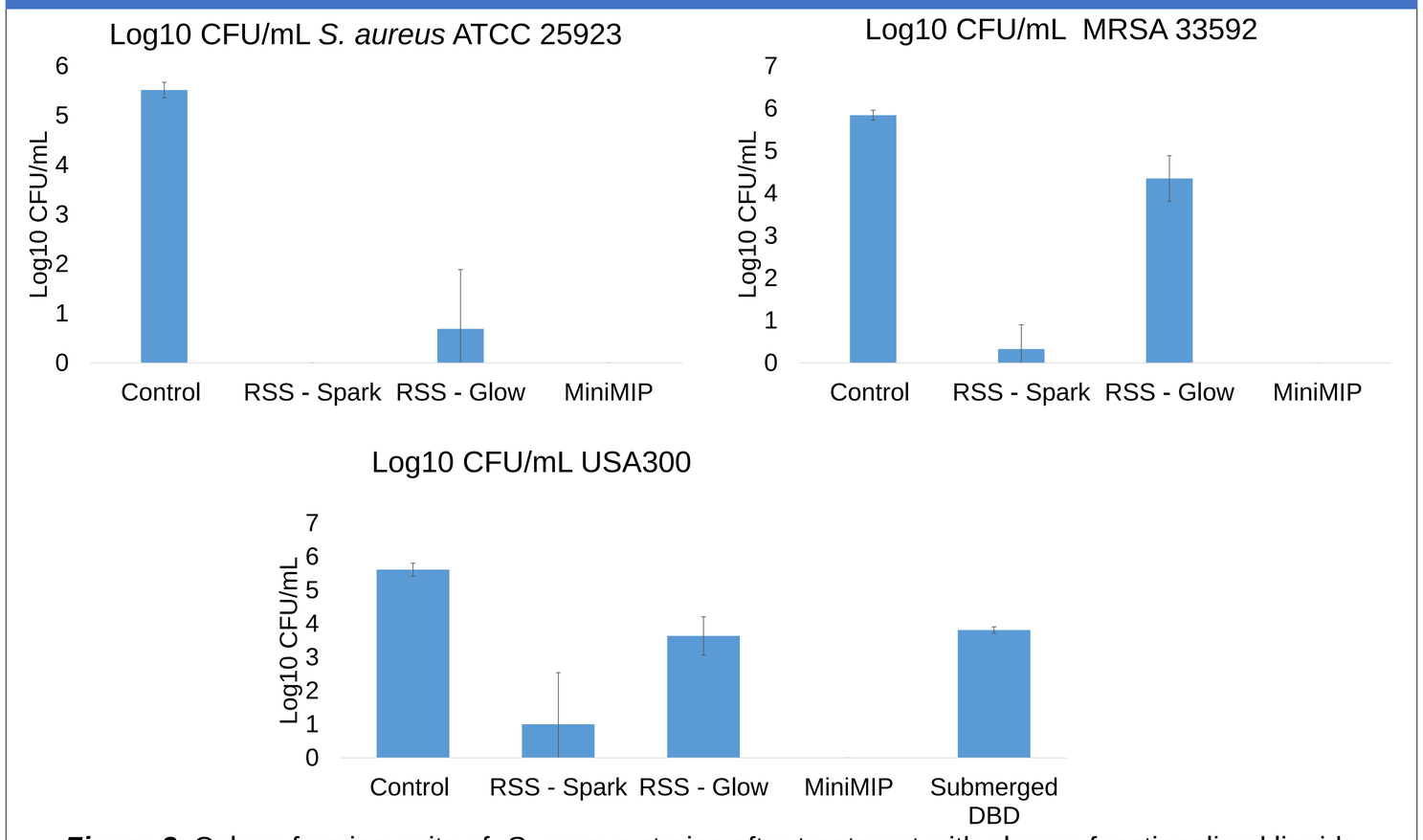

Figure 3. Colony forming units of *S. aureus* strains after treatment with plasma functionalised liquids generated for from the RSS Spark (CT: 15min) , RSS Glow (CT: 30min), MiniMIP (CT:45 sec), and submerged DBD (CT: 15min) (USA300 strain only) systems.

Chemistry of Plasma Functionalised Liquids

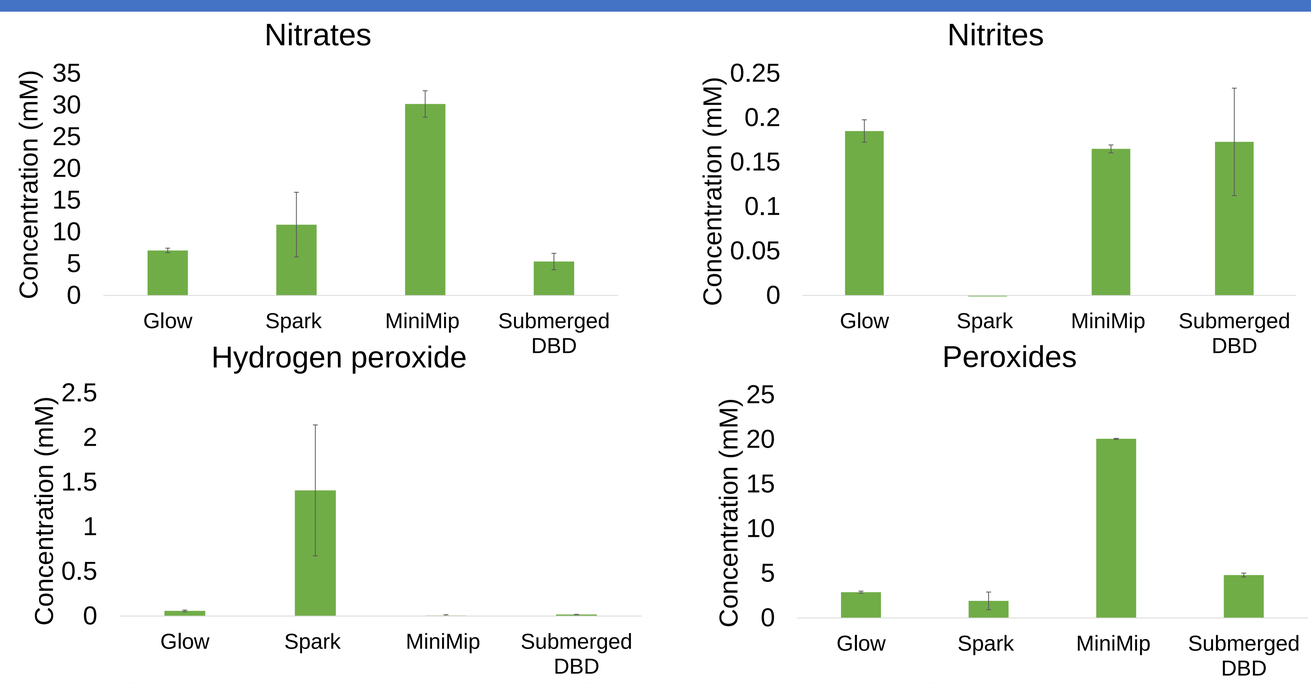

Figure 4. Concentration of Nitrates, Nitrites, Peroxides and Hydrogen Peroxide in plasma functionalised liquids generated by the RSS Glow, RSS Spark, MiniMIP, and Submerged DBD systems.

Plasma functionalised liquids antimicrobial activity against P. aeruginosa

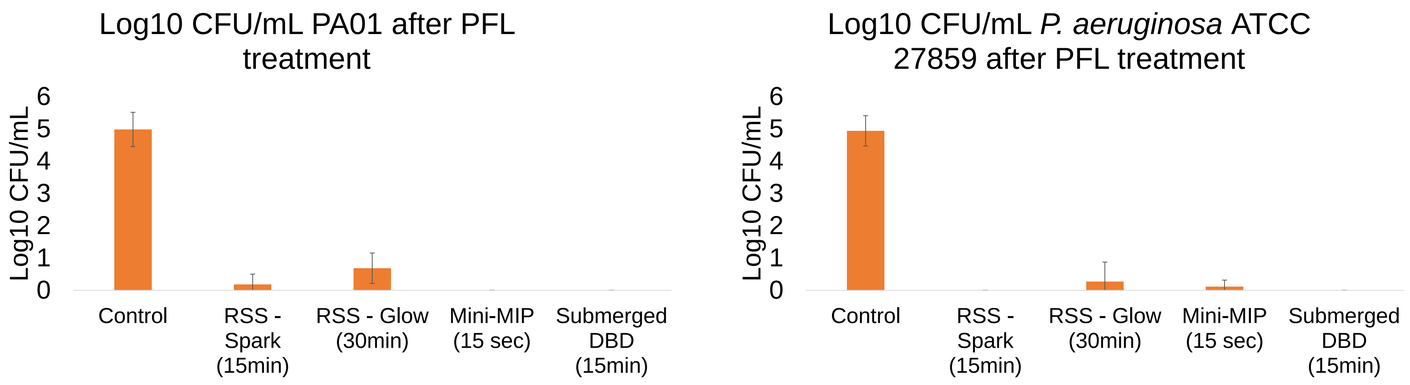

Figure 2. Colony forming units of *P. aeruginosa* strains after treatment with plasma functionalised liquids generated for from the RSS Spark (CT: 15min) , RSS Glow (CT: 30min), MiniMIP (CT:15 sec), and submerged DBD (CT: 15min) systems.

Discussion and Conclusions to Date

- MiniMIP efficacy can be attributed to its acidity and concentrations of NO_3^- which cause oxidative stress leading to DNA damage of Gram positive and cell membrane damage of Gram negative bacteria[2]
- The MiniMIP liquids were the most effective against all bacterial strains
- The method of generating PFLs affects their composition and efficacy

References

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[2] U. Schnabel, M. Balazinski, R. Wagner, J. Stachowiak, D. Boehm, M. Andrasch, et al. 2021. Optimizing the application of plasma functionalised water (PFW) for microbial safety in fresh-cut endive processing. Innovative Food Science & Emerging Technologies 2021 Vol. 72 Pages 102745

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