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Mechanistic Insights to Cold Plasma Functionalised Liquids: Antimicrobial efficacy and Interactions with Processing and Storage Conditions

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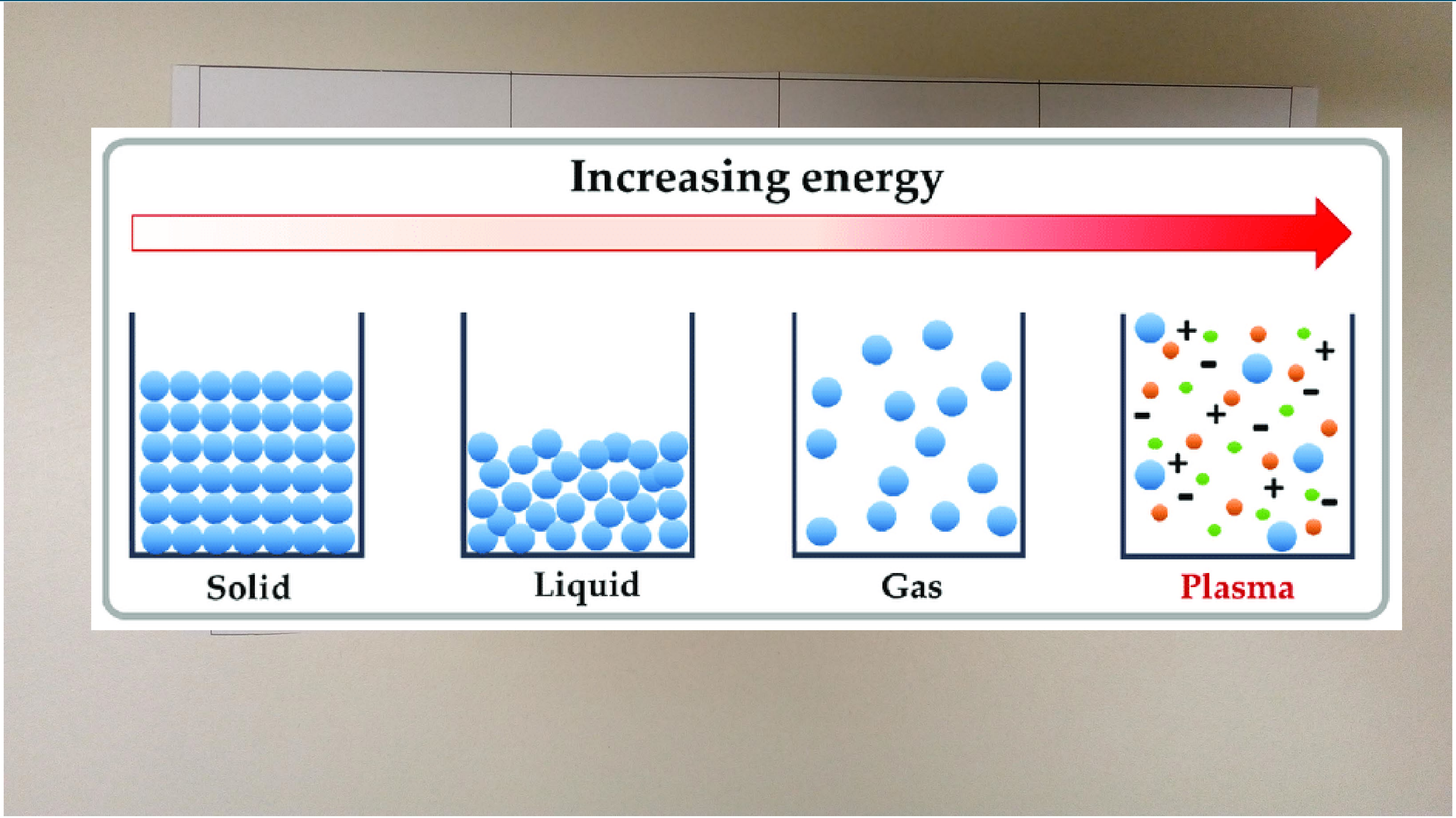
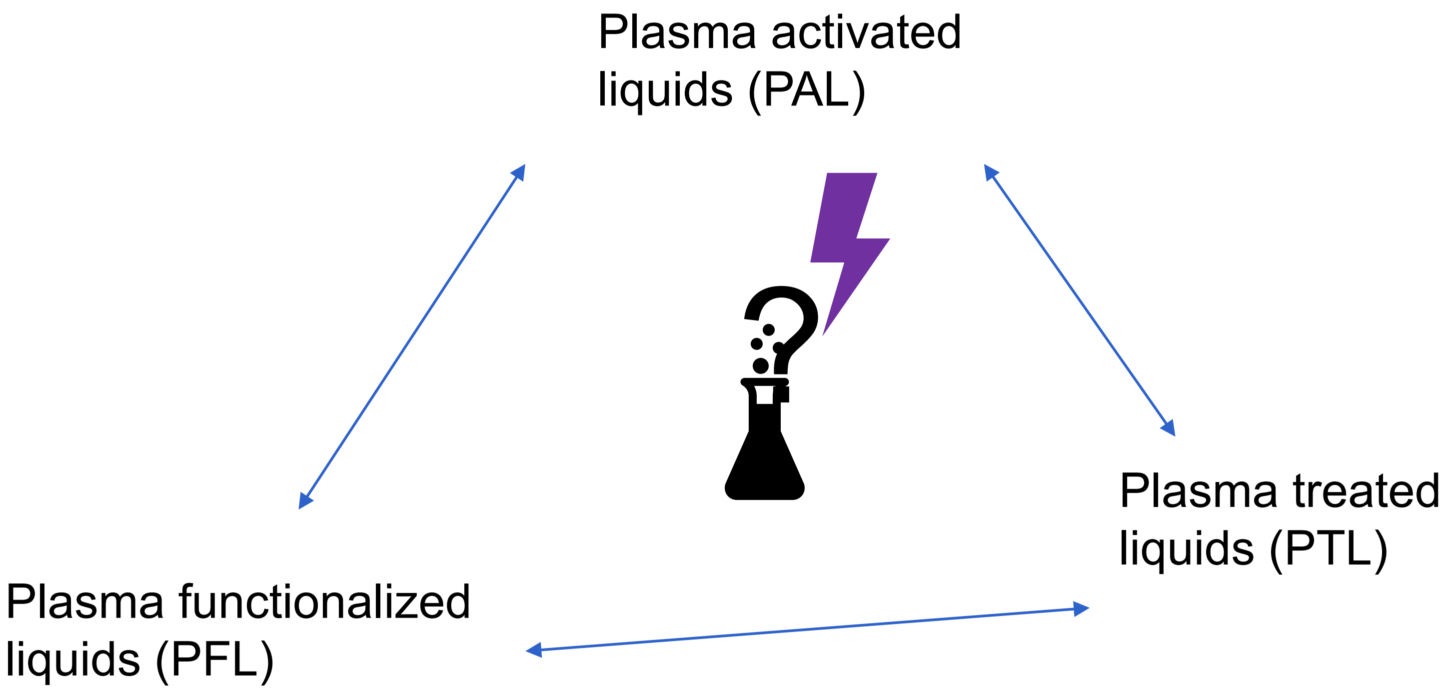
IAFP'S EUROPEAN SYMPOSIUM ON FOOD SAFETY

Plasma

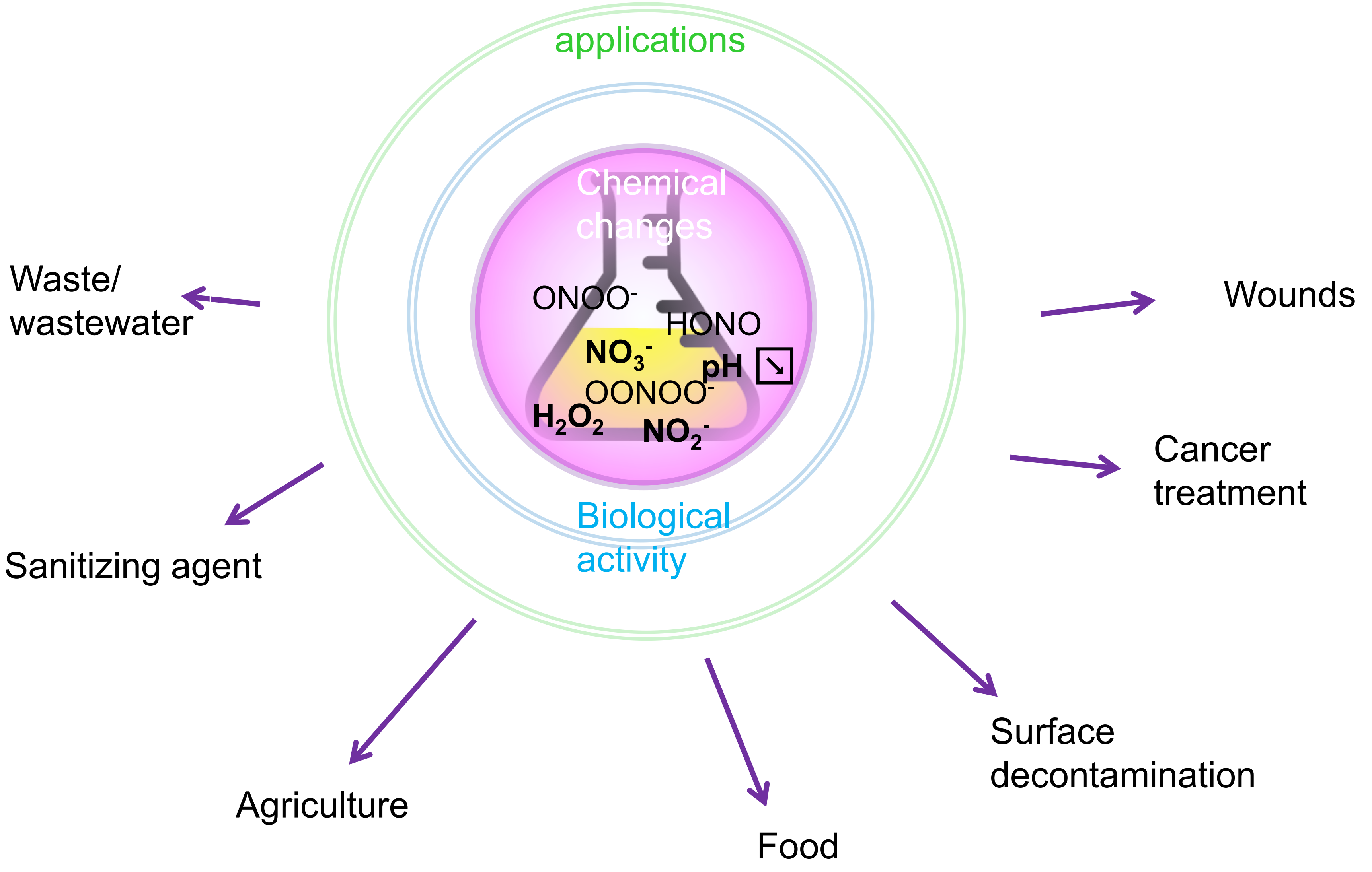
Plasma – the 4th state of matter



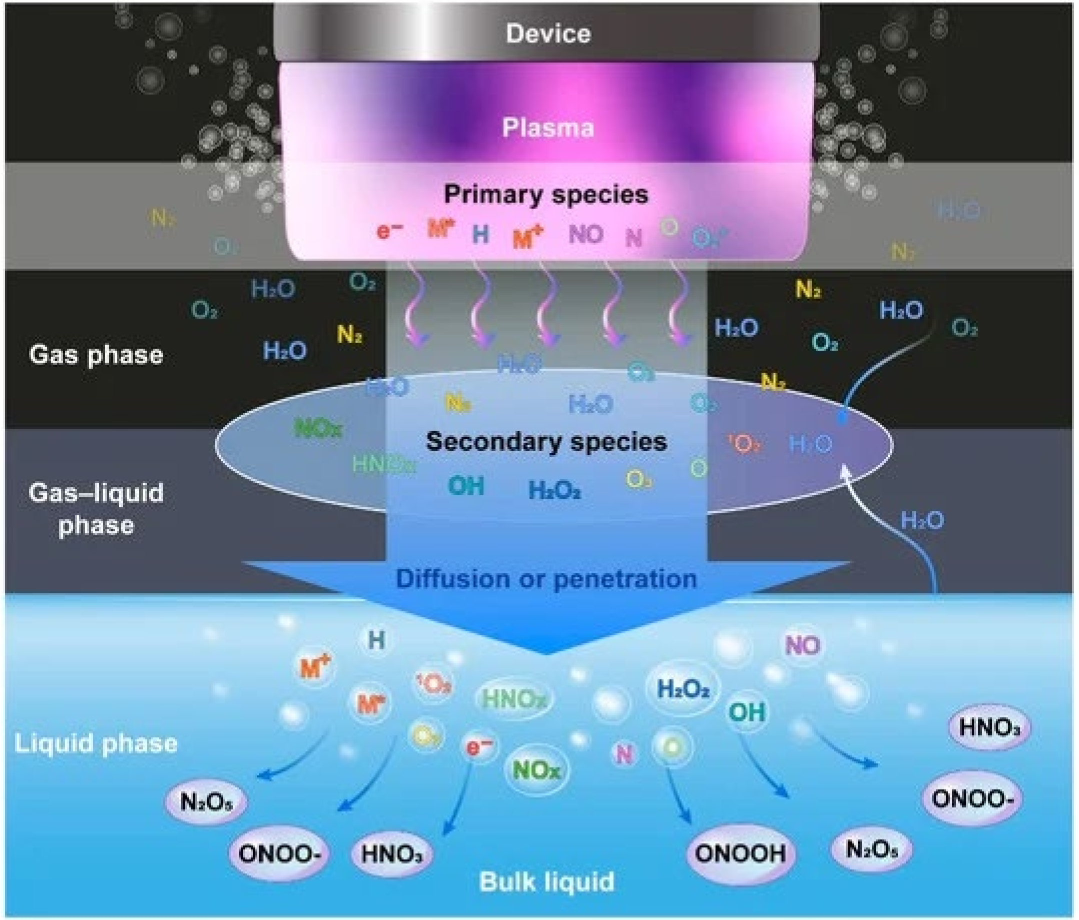
Plasma treatment of liquids



Plasma functionalized liquids (PFL)

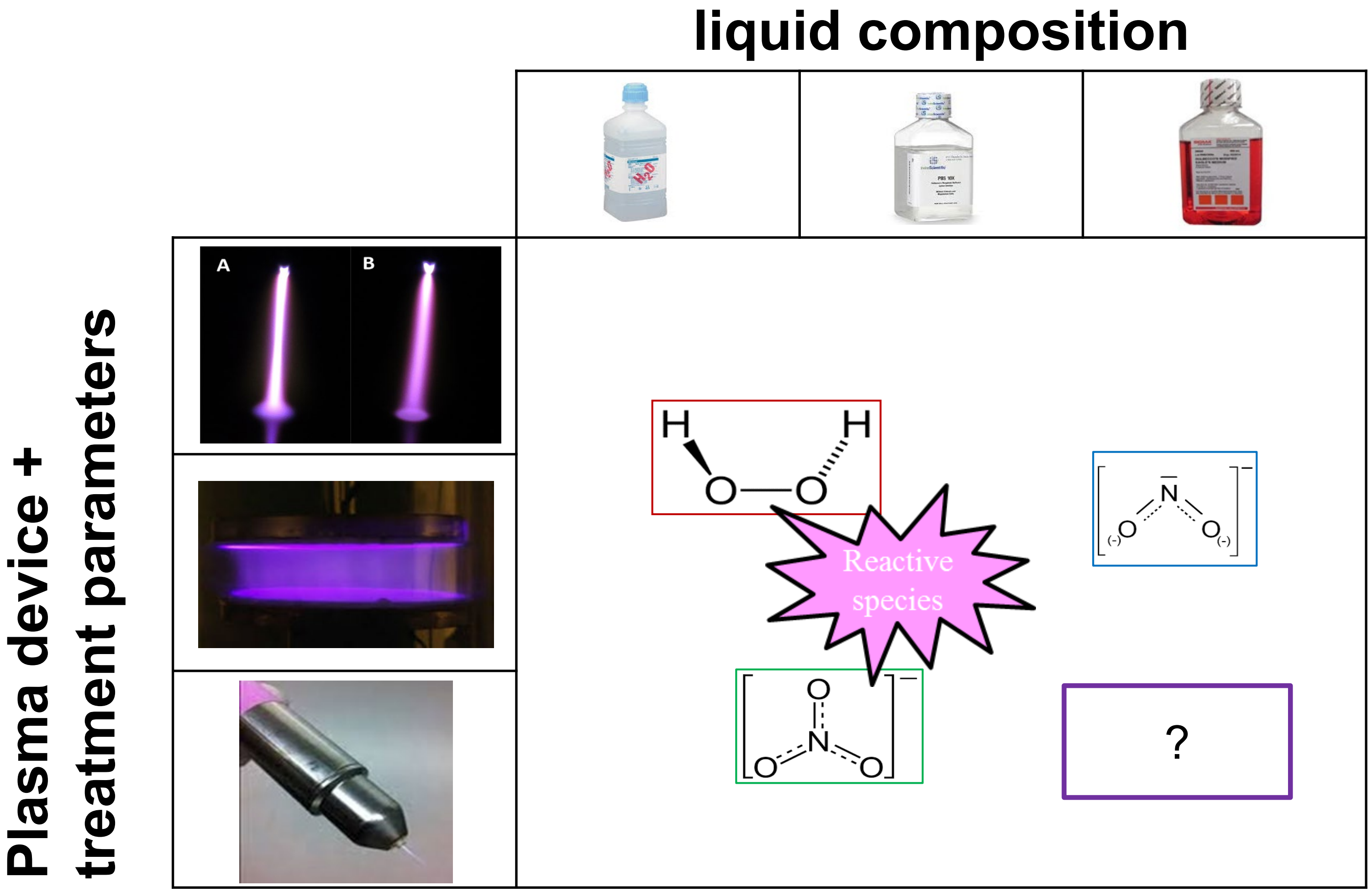


What happens during plasma treatment of liquids?

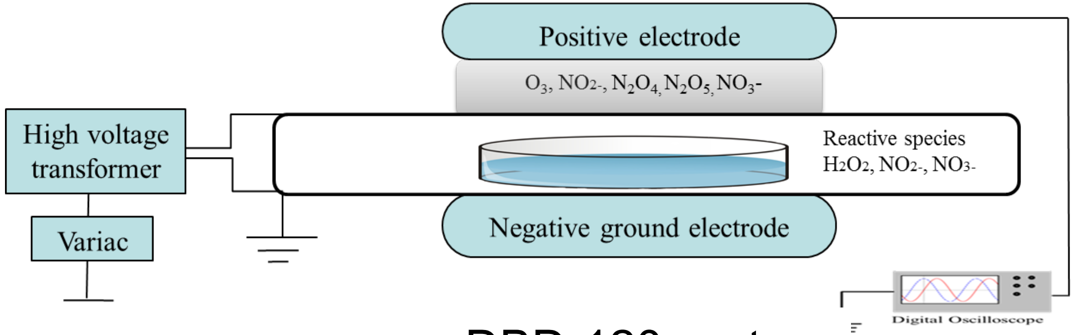


Schematic diagram of formation of reactive species in liquid
(Kim, S.; Kim, C.-H. Biomedicines 2021, 9, 1700. https://doi.org/10.3390/biomedicines9111700)

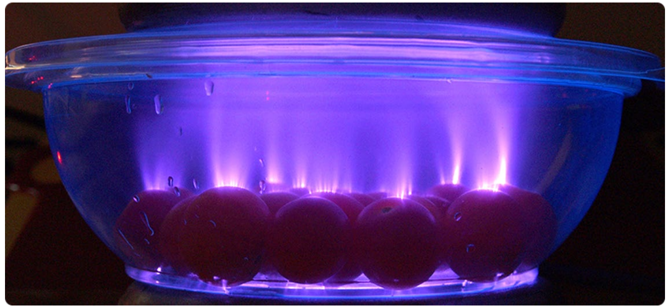
Different plasma functionalized liquids

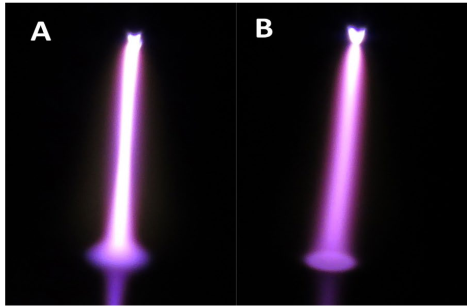


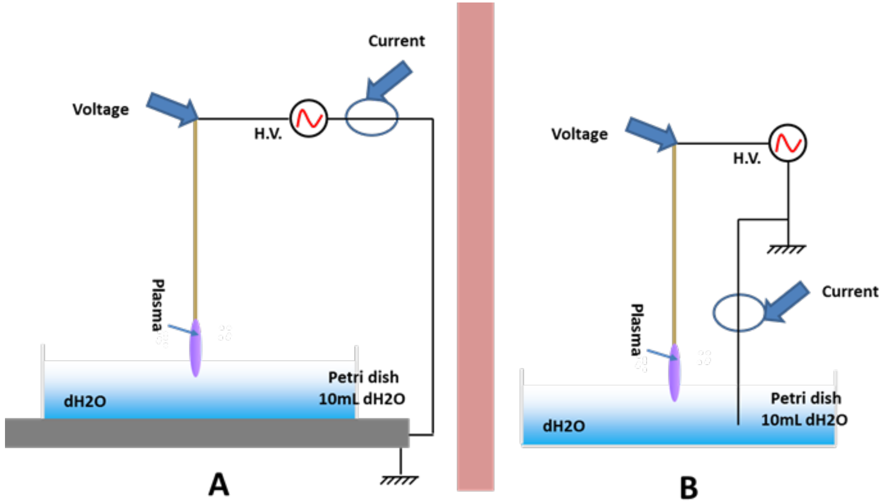
Plasma functionalized liquids based on discharge in air



DBD-120 system

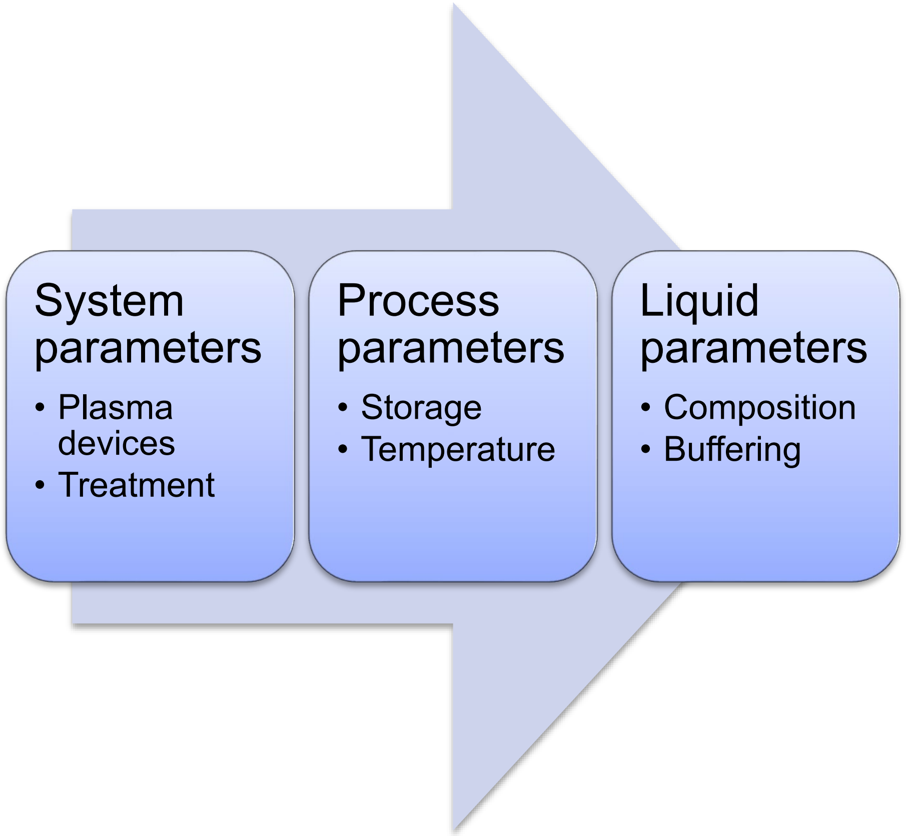






RSS system

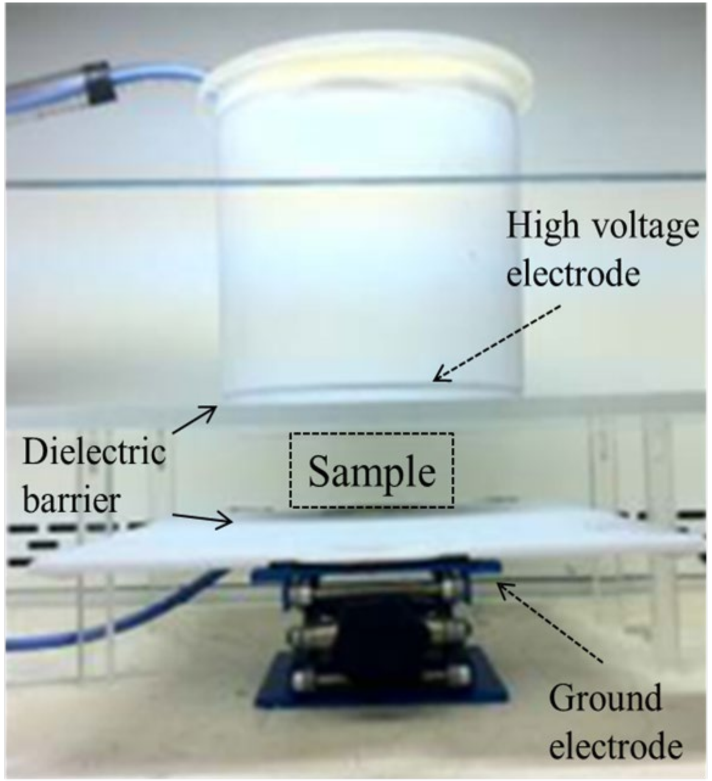
Reactive chemical species in plasma functionalized liquids



- Chemistry:
  - pH, ORP, conductivity
  - Detection of long-lived ROS/RNS
    - H_2O_2: $TiOSO_4$
    - Oxidative species (peroxides, HNO_2): KI (buffered/non-buffered)
    - NO_2^-: Griess
    - NO_3^-: Dimethylphenol

Tsoukou et al. (2018), *Plasma medicine*
Niquet, Boehm et al. (2017), *Plasma Processes and Polymers*

The DBD120 system

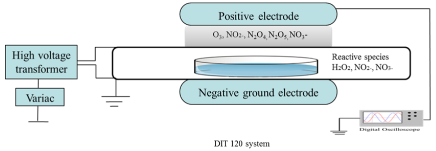


Liquid composition

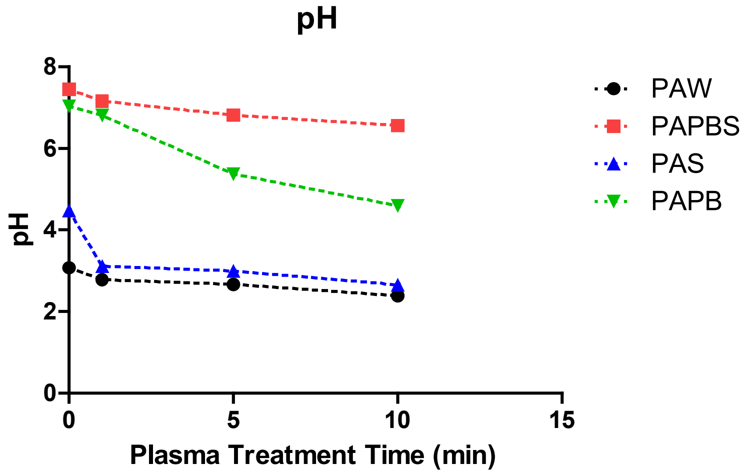
		Buffered (KH_2PO_4/K_2HPO_4)	
		-	+
Saline (NaCl)	-	-/- H_2O	-/+ PB
	+	+/- S	+/+ PBS

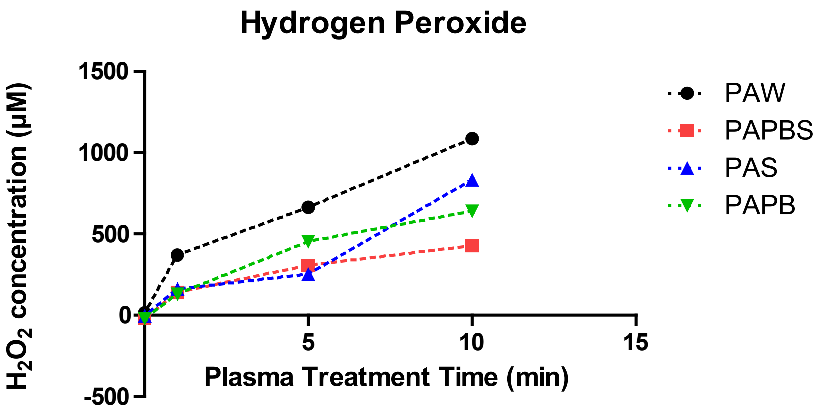
Voltage: 0-120kV
Frequency: 50 Hz
Gap: 22mm

Tsoukou et al. (2018), *Plasma medicine*



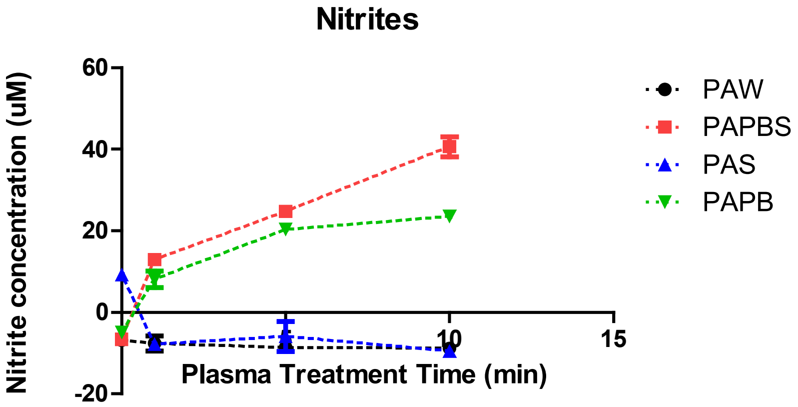
Chemical characterization

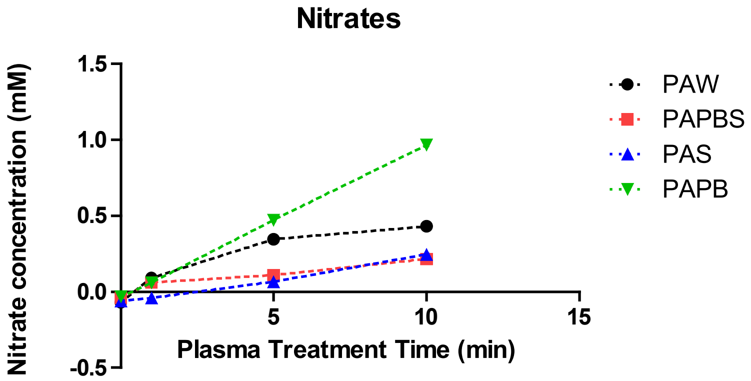


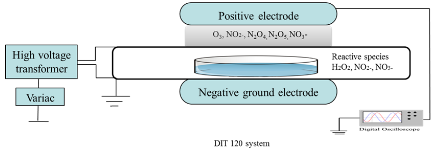


Evanthia Tsoukou

PAW: Plasma activated Water
PAPBS: Plasma activated PBS
PAS: Plasma activated Saline
PAPB: Plasma activated PB

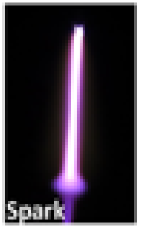


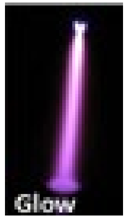




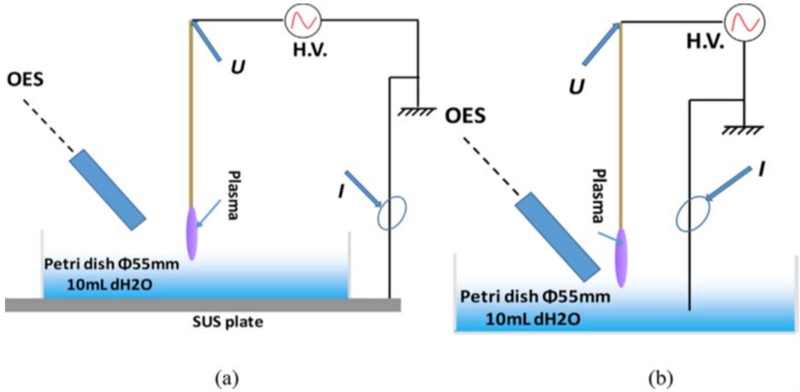
The RSS plasma system

Dr. Peng Lu



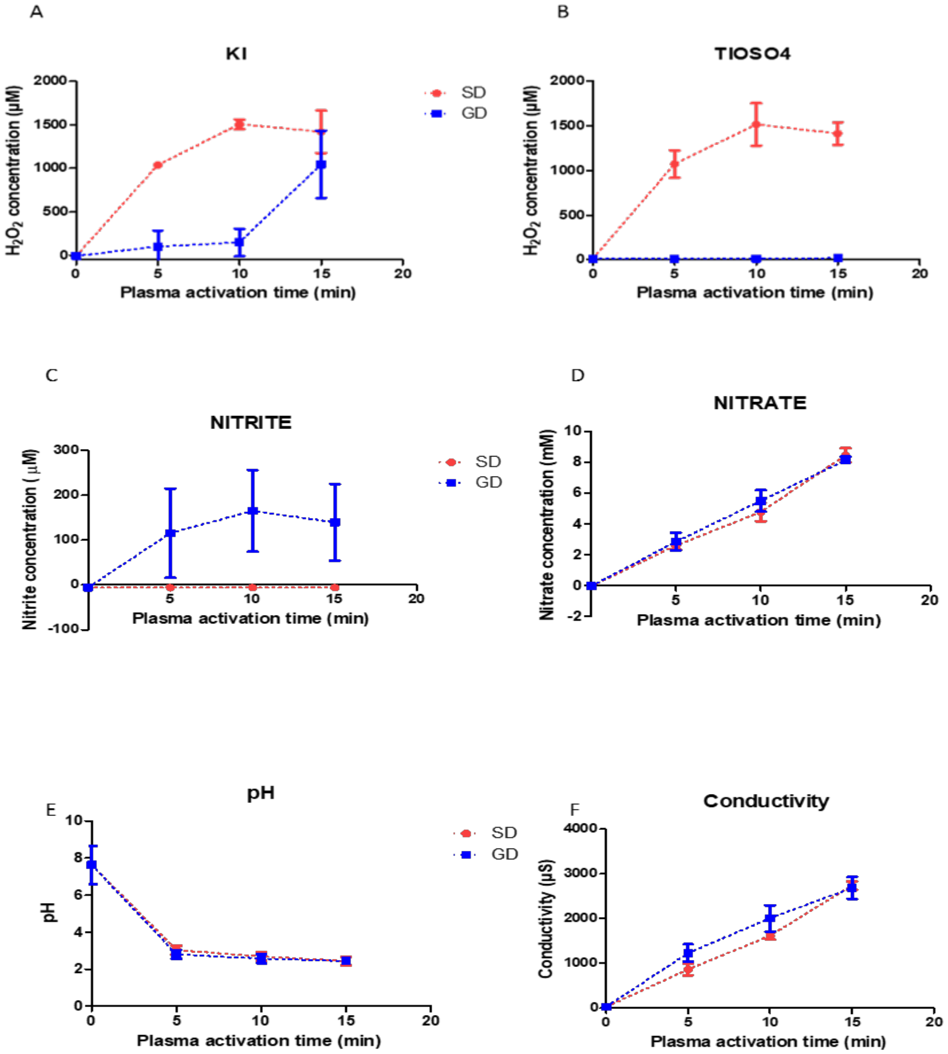


Spark (SD) Glow(GD)



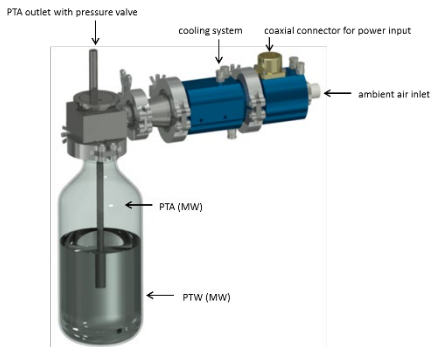
ROS-rich
H_2O_2, NO_3^-

RNS-rich
NO_3^- NO_2^-

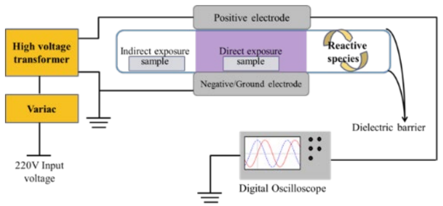


Lu et al. (2017) *Plasma Processes and Polymers*

Different plasma systems – different chemistry

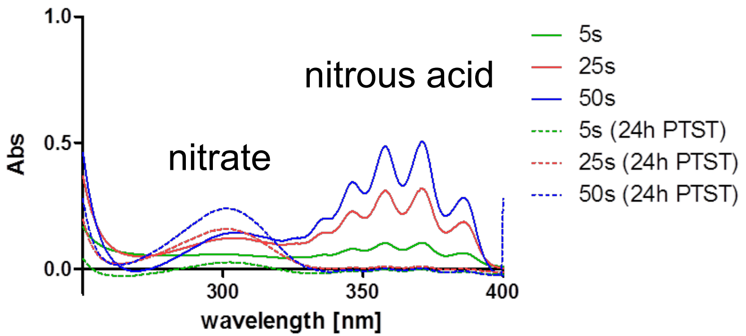


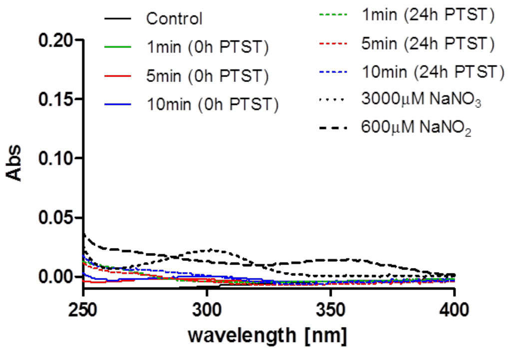
MW plasma



DBD plasma

	PTW-MW	PTW-DBD
Input energy	90-920 W min	300-3500 W min
Nitrous acid	2-12 mM	Not detected
Nitrite	2-20 mM	Not detected
Nitrate	1-25 mM	0.1-0.8 mM
Hydrogen peroxide	Not detected	0.02-0.4 mM
Contact time for microbial inactivation	~1 min	~60 min





Collaboration between TU Dublin and the INP Greifswald:

Comparison of **chemical composition** and **antimicrobial efficacy** of plasma activated water

Niquet, R. et al. (2017), Characterising the impact of post-treatment storage on chemistry and antimicrobial properties of plasma treated water derived from microwave and DBD sources. *Plasma Processes and Polymers.* DOI: 10.1002/ppap.201700127.

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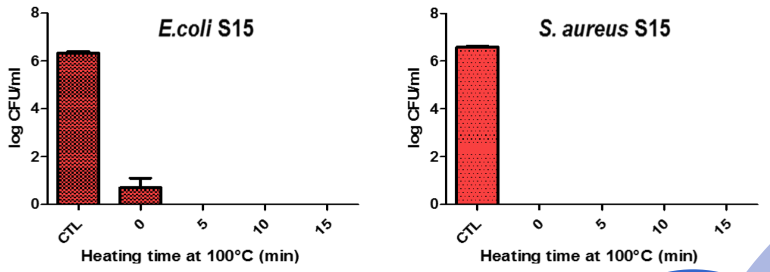
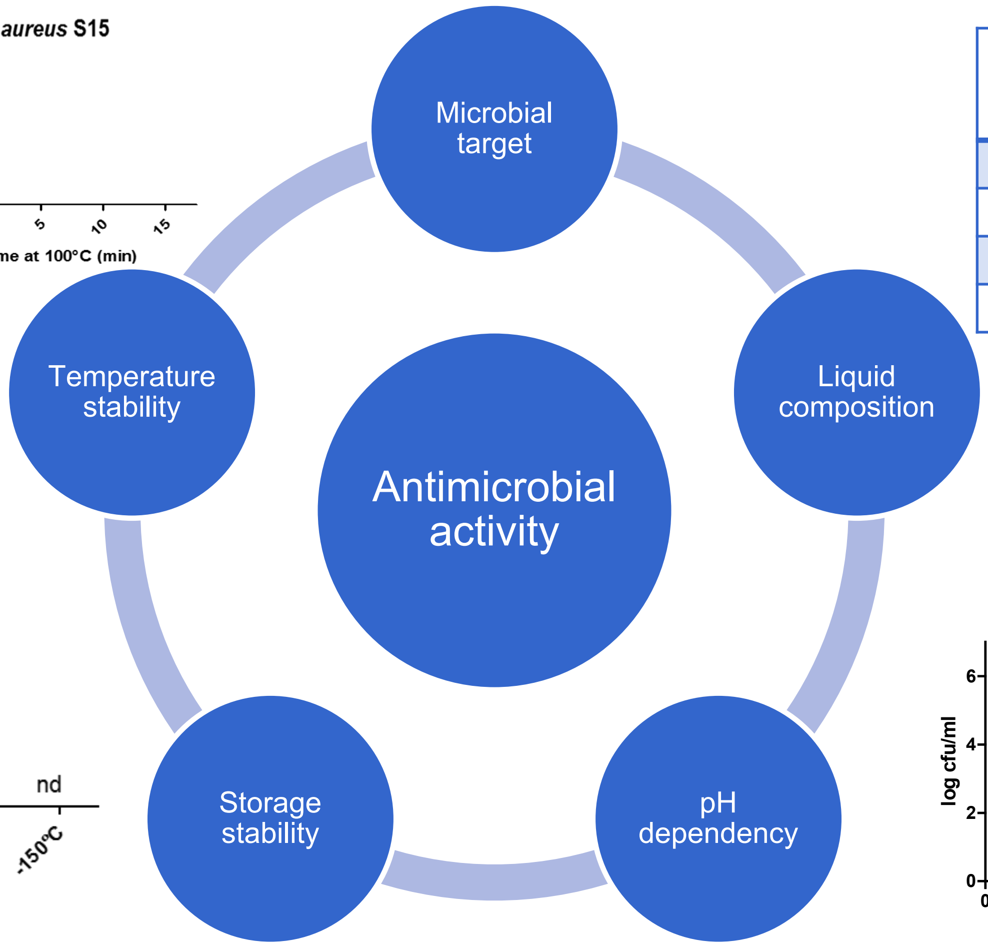
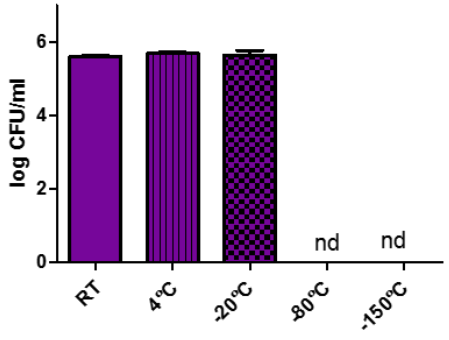
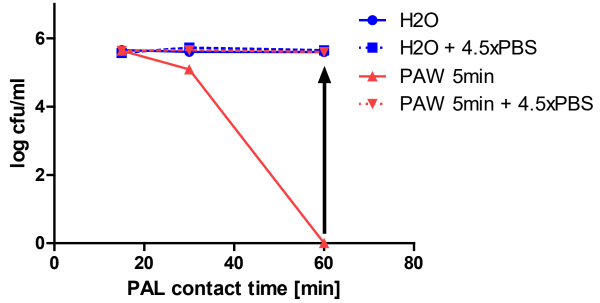
Effects of PFLs on prokaryotic cells





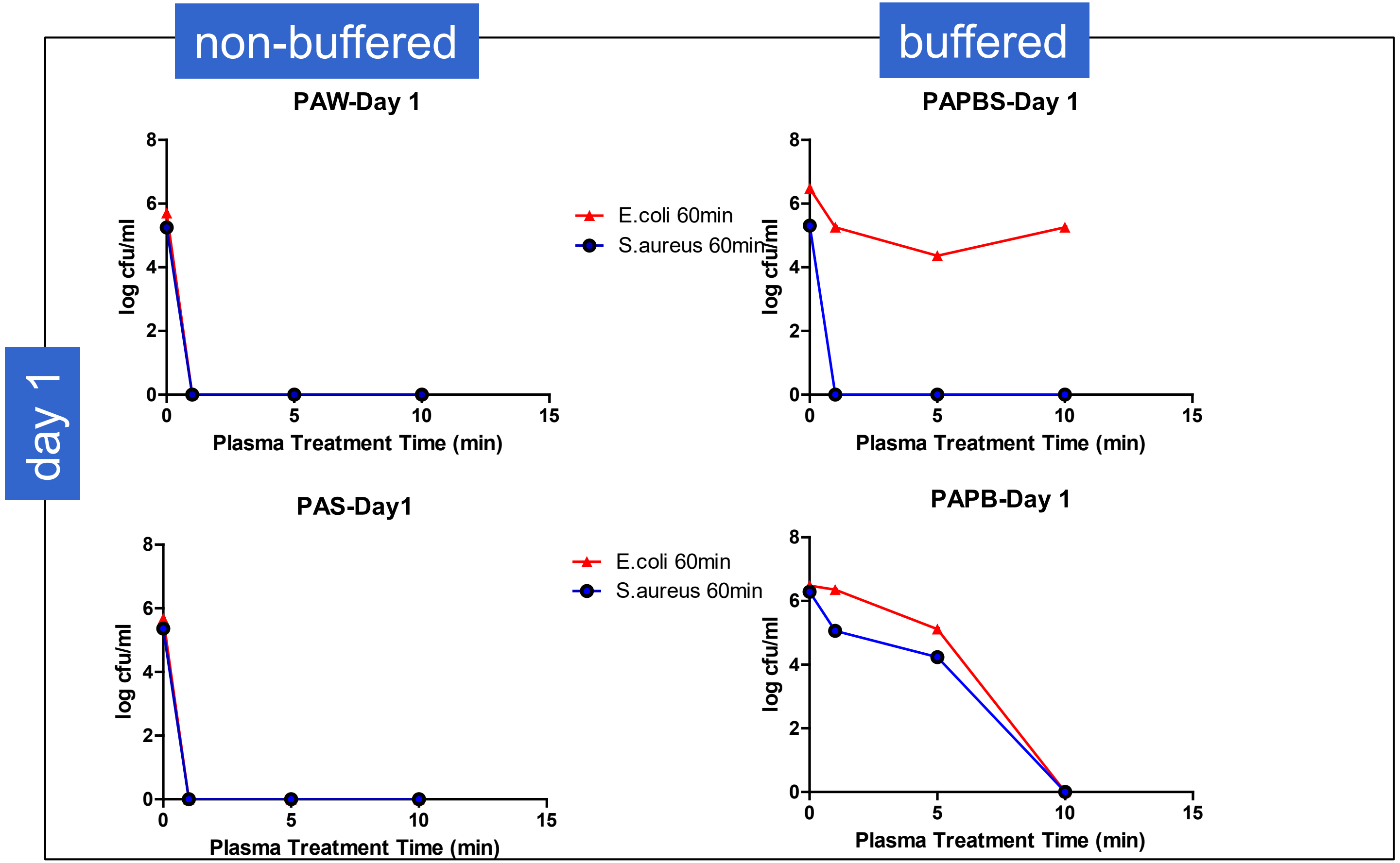
PAL	Antimicrobial Efficacy (E.coli/S.aureus)
PAW	Strong/Strong
PAPBS	Weak/Strong
PAS	Strong/Strong
PAPB	Median/Median

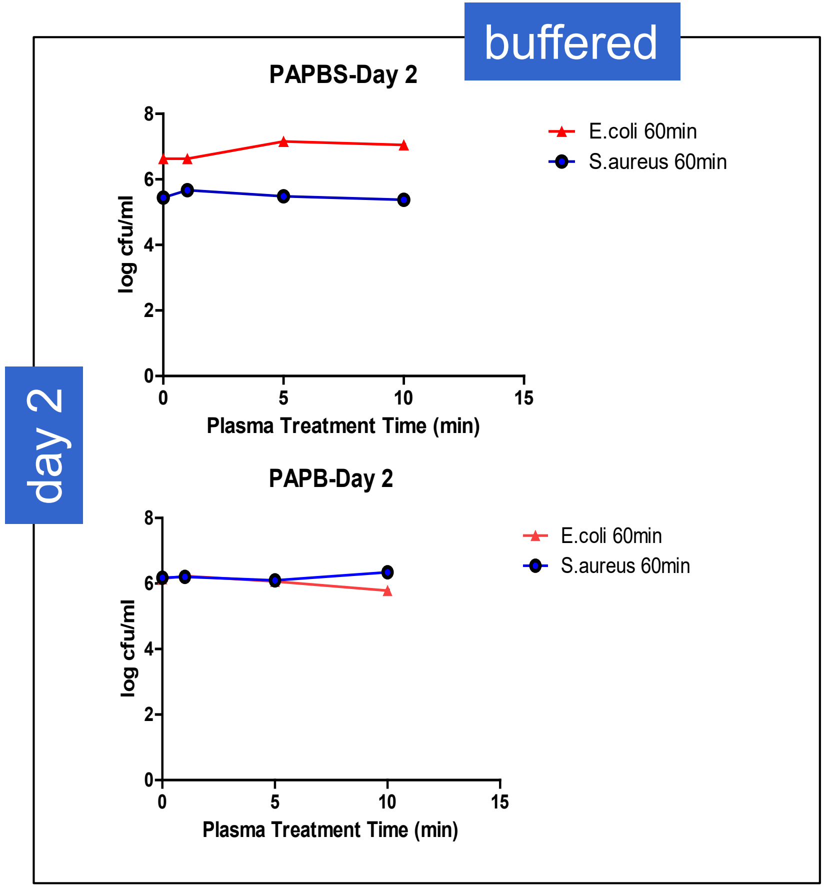


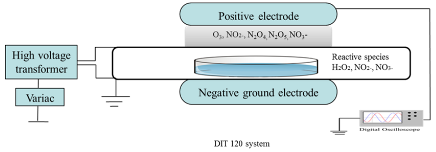


Tsoukou, E.; Bourke, P.; Boehm, D. (2020). Water, 12, 3021.
https://doi.org/10.3390/w12113021.
Tsoukou, E.; Delit, M.; Treint, L.; Bourke, P.; Boehm, D. (2021). Appl. Sci., 11, 1178.
https://doi.org/10.3390/app11031178

Antimicrobial activity and stability of PFLs

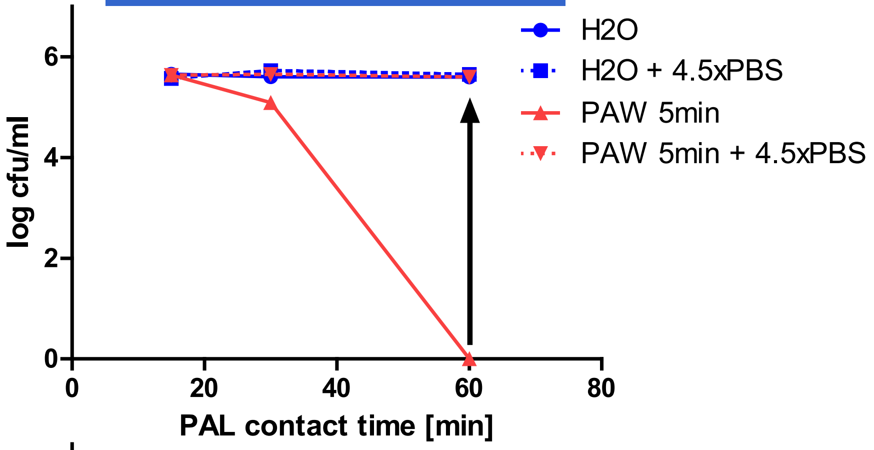


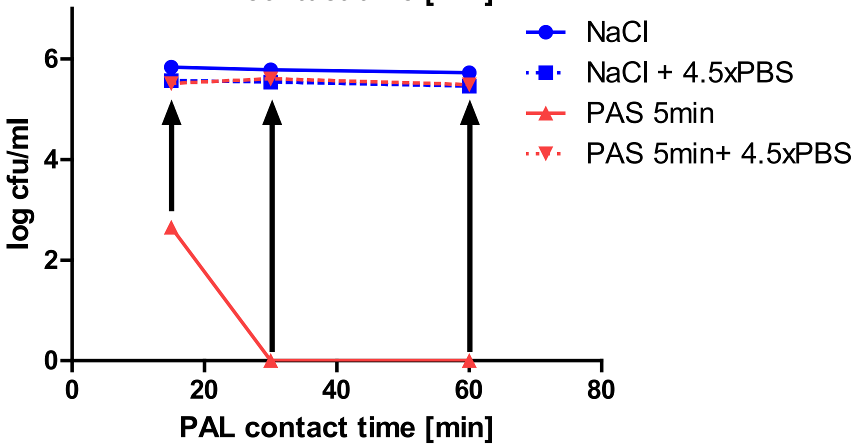




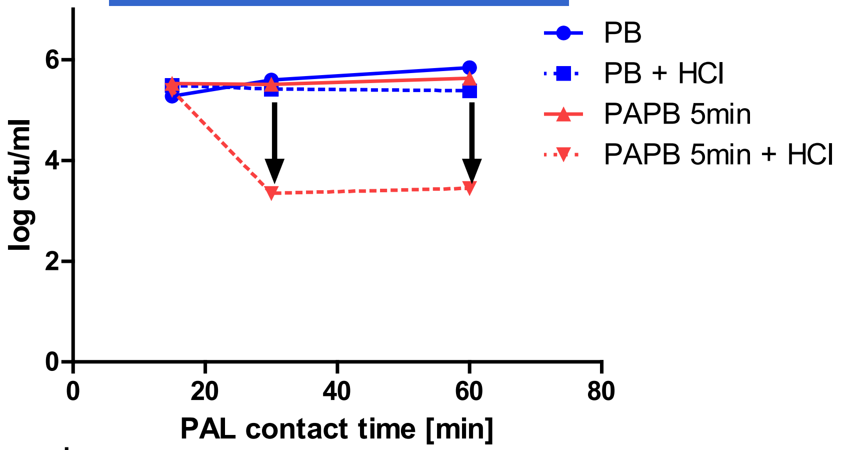
The role of pH in PFL antimicrobial activity

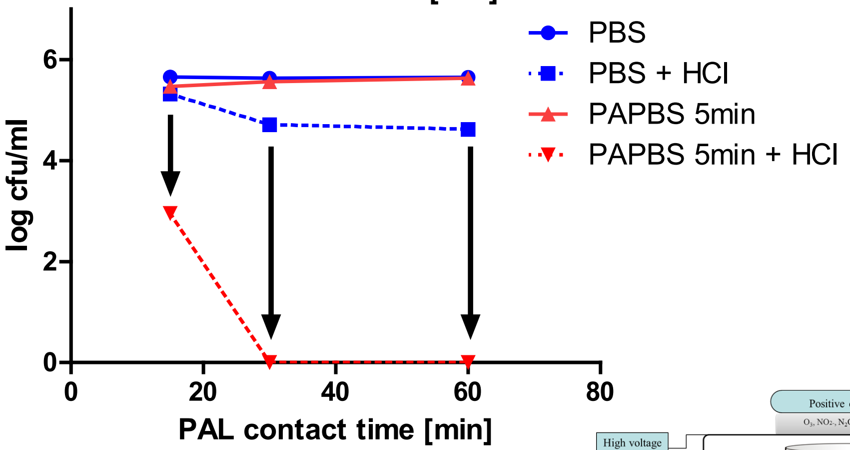
Neutralization of pH

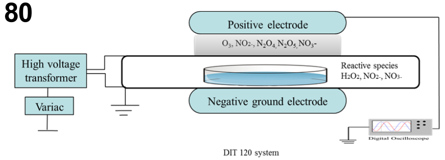




Reduction of pH







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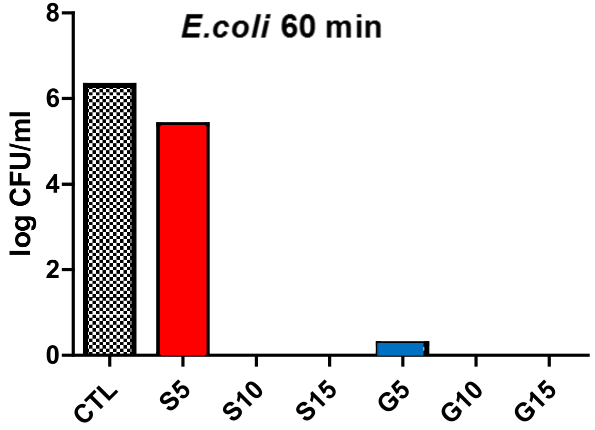
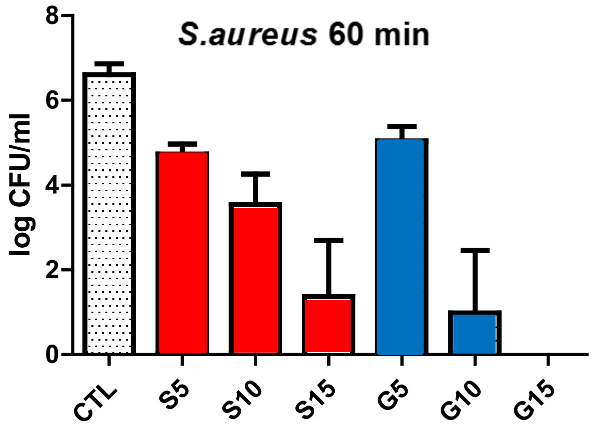
Antimicrobial activity and stability (RSS system)

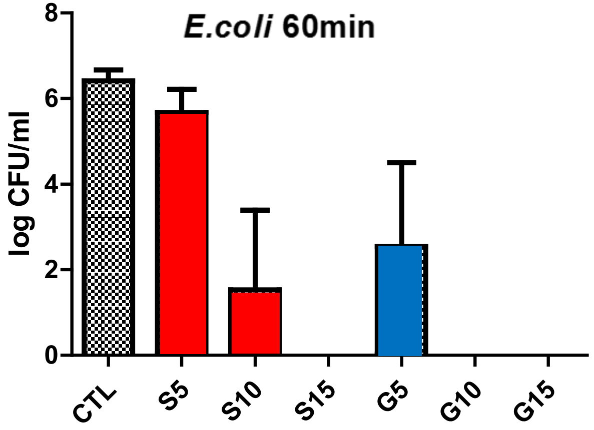
PFW:
Spark (S) 5, 10, 15min
Glow (G) 5, 10, 15min

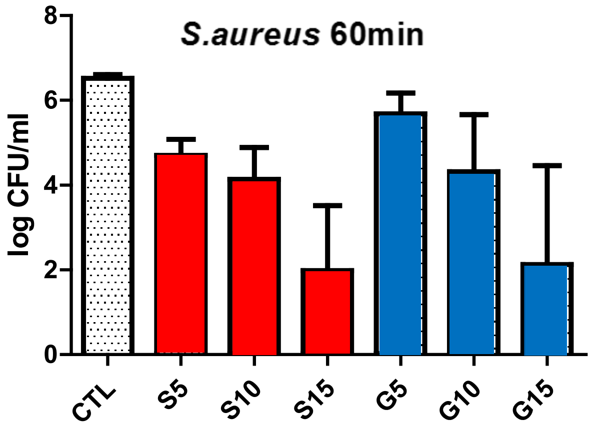
Day 1

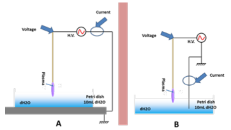




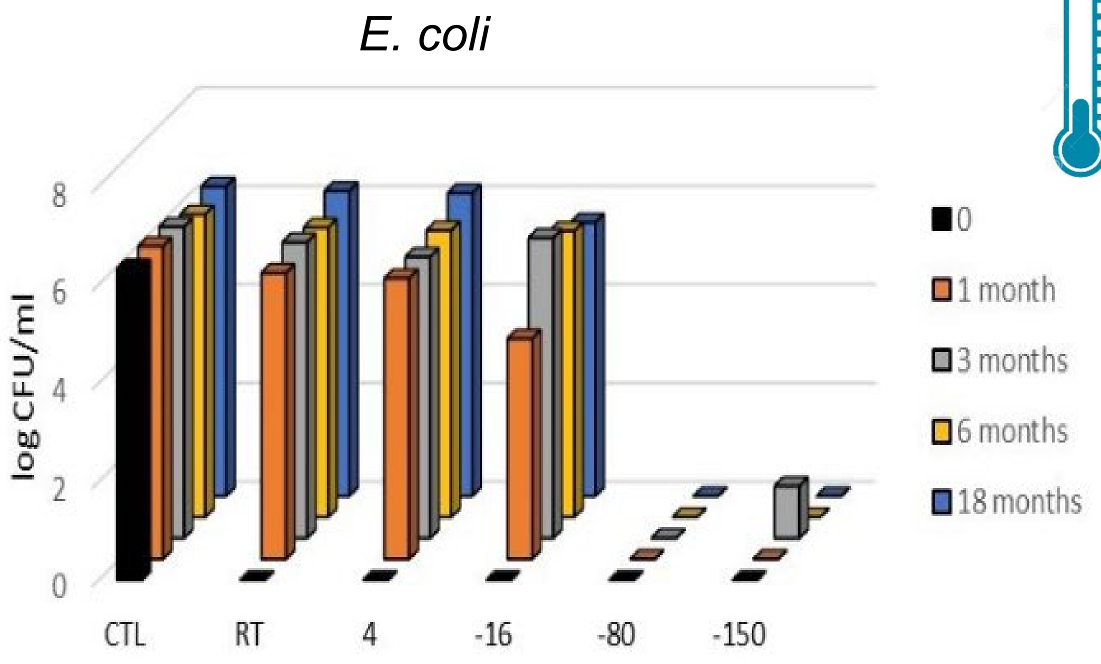
1 Week

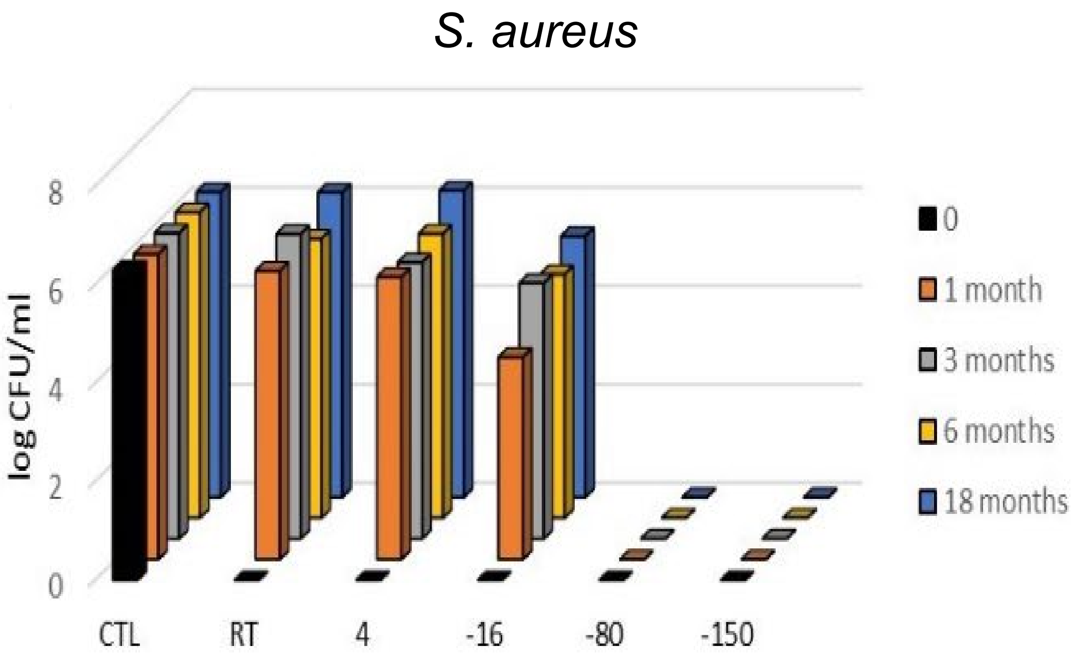






Temperature stability

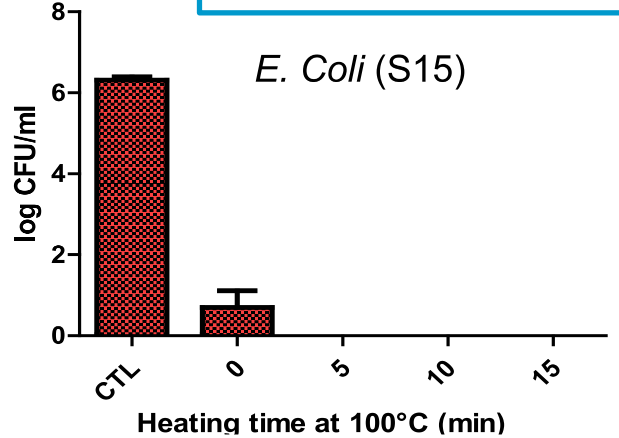




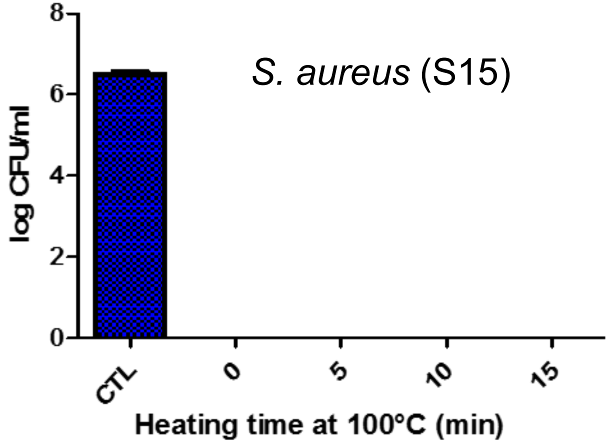
Bactericidal effects retained after prolonged storage at -80, -150°C

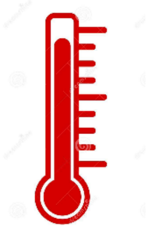
Stability at high temperature and pressure

PFW heated at 100°C

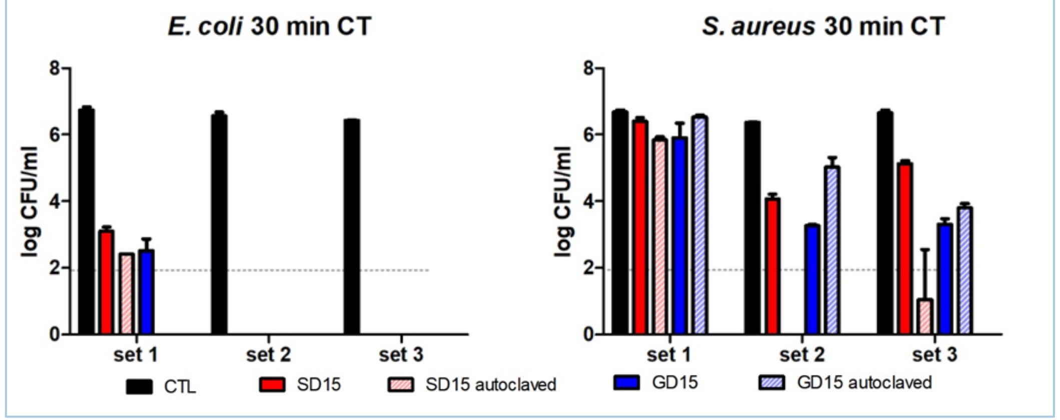


- Retention of antimicrobial efficacy at high temperature



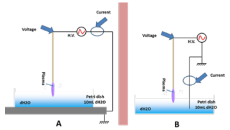


PFW autoclaved at 121°C



- Enhanced antimicrobial efficacy at high temperature and pressure?

Tsoukou E., et al (2021). Distinct Chemistries Define the Diverse Biological Effects of Plasma Activated Water Generated with Spark and Glow Plasma Discharges. *Applied Sciences*

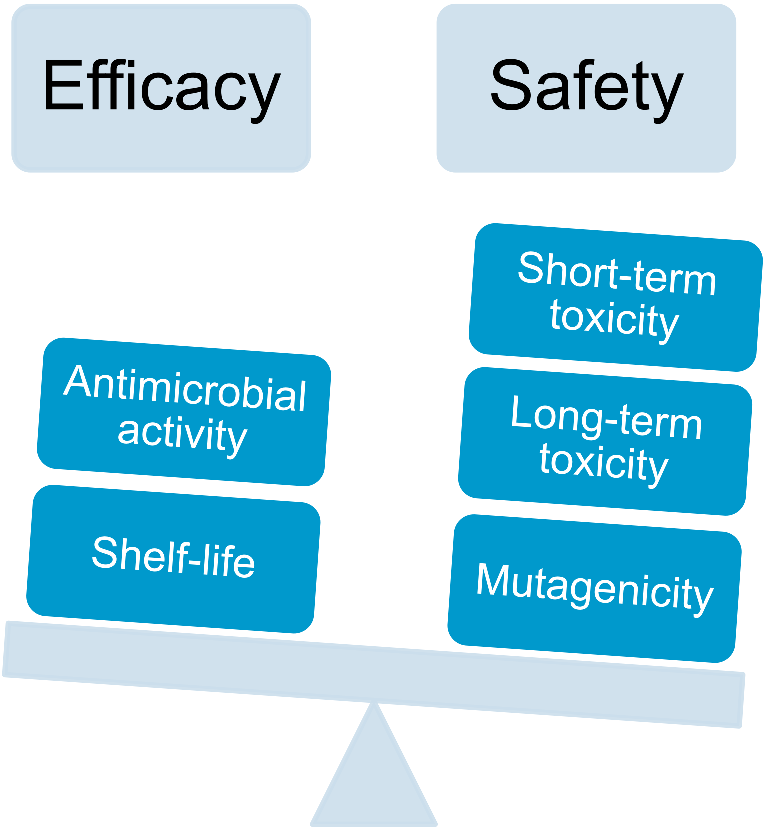


Stability of PFL

Why does it matter?

- Off-site production
- Storability
- Applications in fumigation/vapourization
- Understanding chemistry and secondary reactions

Safety of plasma activated liquids



Short and long-term safety

Cytotoxicity testing

- Mammalian cell models
- *Galleria melonella*

Genotoxicity testing

- Mammalian cell model (HPRT assay)
- Bacterial cell model (AMES test)

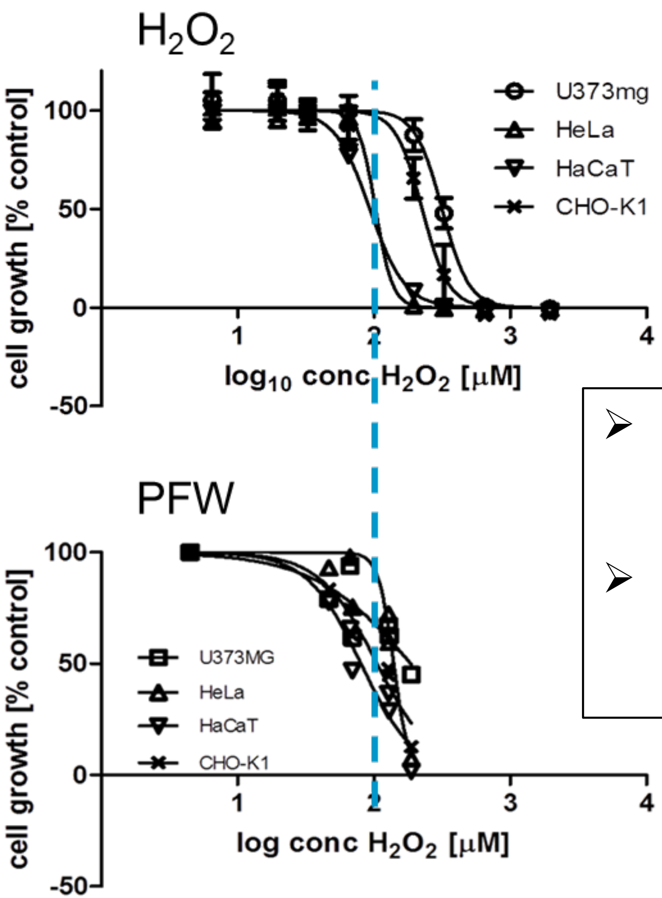
Long-term exposure – mutagenic effects

FBS		Day in culture									
		0	3	8	11	15	18	22	29	32	39
Control	A	-	-	-	-	-	-	-	-	-	+
	B	-	-	-	-	-	-	-	-	-	-
	C	-	nd	-	-	-	-	+	-	-	+
1 min	A	nd	-	-	-	-	-	-	-	+	+
	B	nd	-	+	-	-	-	+	-	+	-
	C	nd	+	-	-	-	-	+	-	-	+
5 min	A	nd	-	-	+	-	-	+	-	-	+
	B	nd	+	-	-	+	-	-	-	-	-
	C	nd	-	-	-	+	-	+	-	+	+
10 min	A	nd	-	-	-	-	-	-	-	-	+
	B	nd	-	-	-	-	-	-	-	-	+
	C	nd	-	-	-	-	-	-	-	-	-

Highest occurrence of mutations (5 min)

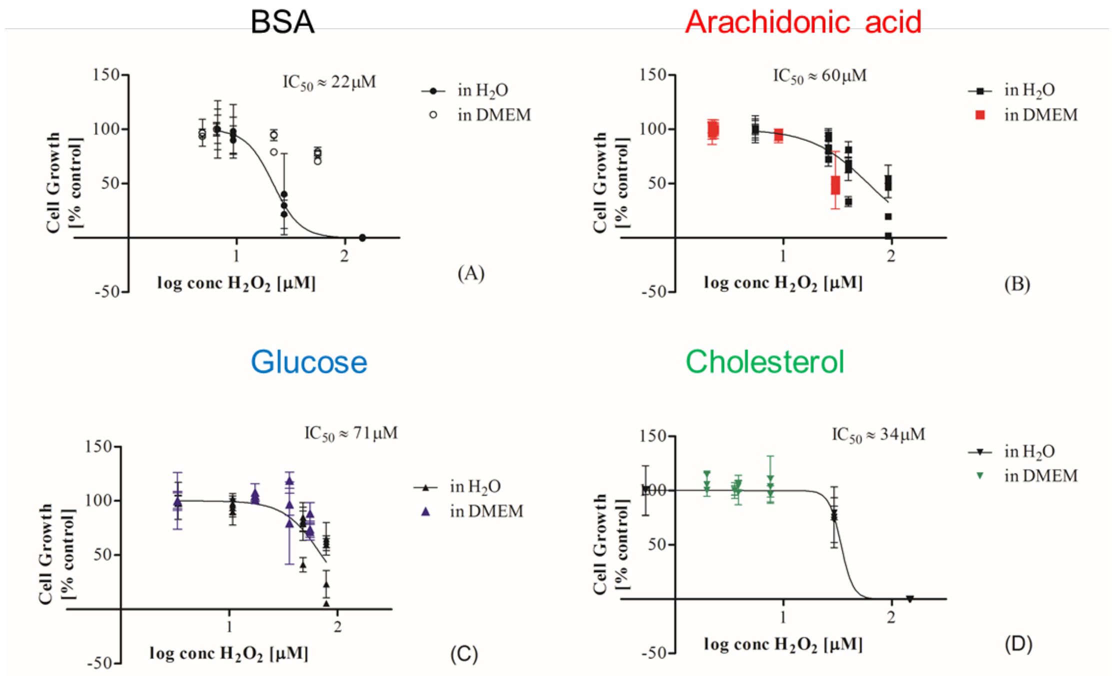
Increasing rate of mutations over time

Cytotoxicity and the role of H_2O_2



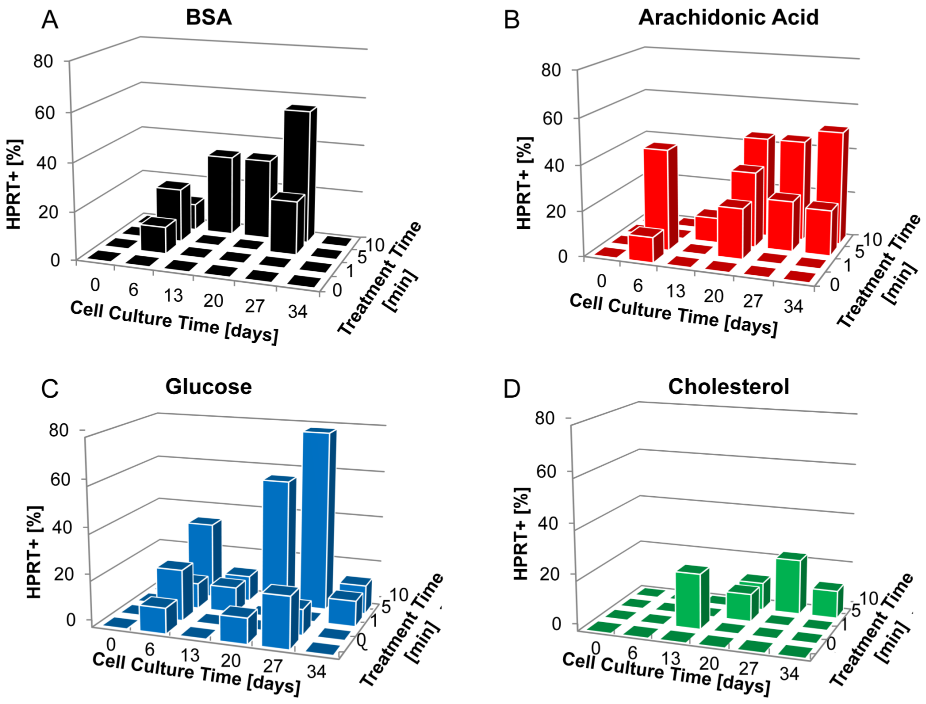
- H_2O_2 in PFL contributes to cytotoxic effects
- other reactive species are involved

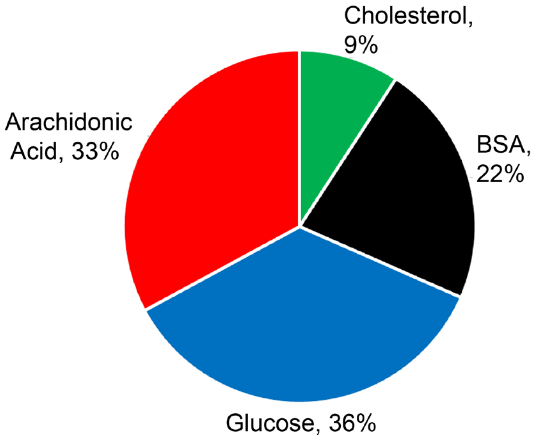
Model biomolecule solutions



- Differences in cytotoxic effects of biomolecule solutions
  - not a result of different H_2O_2 concentrations

Plasma-treated biomolecule solutions – mutagenic potential





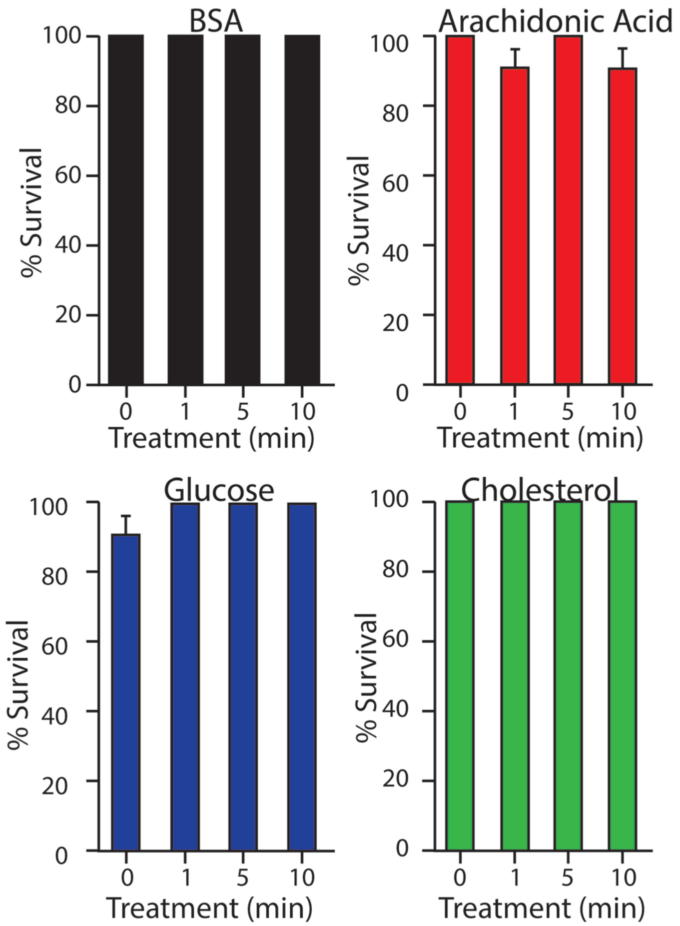
Cell culture medium supplemented with 10% (v/v) biomolecule solution (in DMEM-F12) at each sub-culturing over 34 days

Heslin et al (2021), Biomolecules as Model Indicators of In Vitro and In Vivo Cold Plasma Safety. Front. Phys. 8:613046. doi: 10.3389/fphy.2020.613046

In vivo toxicity testing

Dead larvae

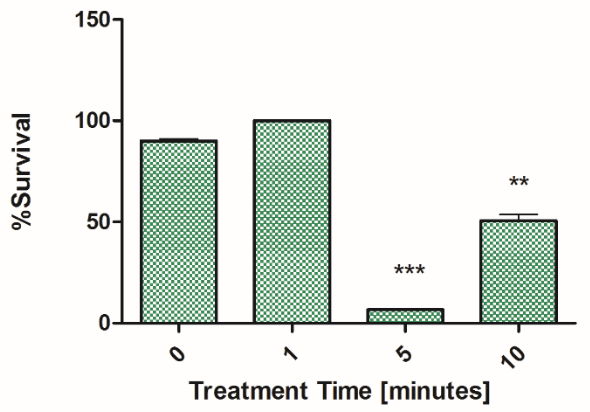
Galleria melonella, injection model

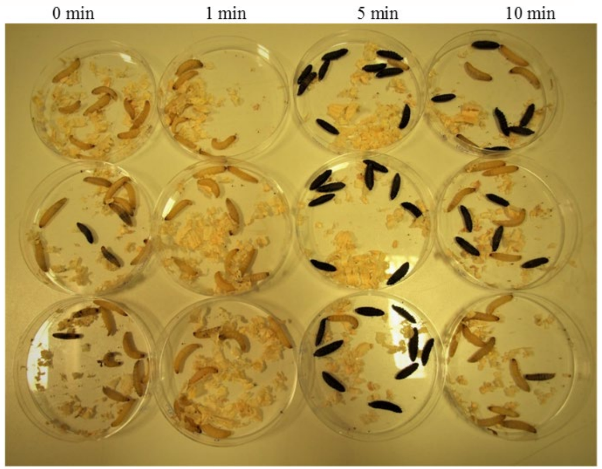


Toxicity testing of a plasma treated food model

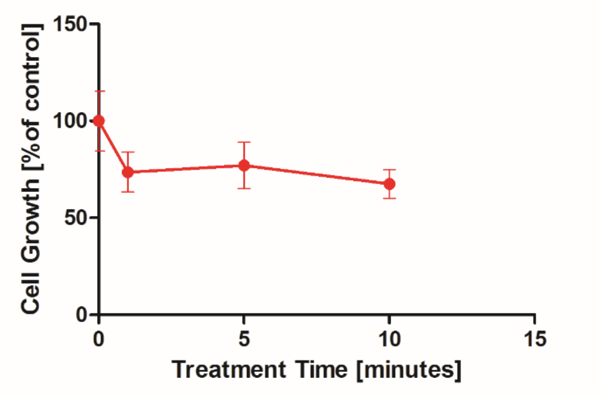
- lettuce broth
  Plasma treatment:
  0, 1, 5, 10 min

Short-term *in vivo* toxicity





Short-term *in vitro* toxicity



long-term *in vitro* mutagenicity

Lettuce Broth		Days in Culture						
		0	6	13	20	27	34	40
Control Untreated	A	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-
	B	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-
	C	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-
1 min	A	nd	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-
	B	nd	-/-/-	-/-/-	-/-/-	-/+/+	-/-/-	-/-/-
	C	nd	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-
5 min	A	nd	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-
	B	nd	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-
	C	nd	-/-/-	-/-/-	-/-/-	-/-/-	-/-/ +	-/-/-
10 min	A	nd	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-
	B	nd	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-
	C	nd	-/-/-	-/-/-	-/-/-	+/+/+	-/-/-	-/-/-

Heslin et al (2020), Safety evaluation of plasma-treated lettuce broth using in vitro and in vivo toxicity models Front. Phys D. DOI: 10.1088/1361-6463/ab7ac8

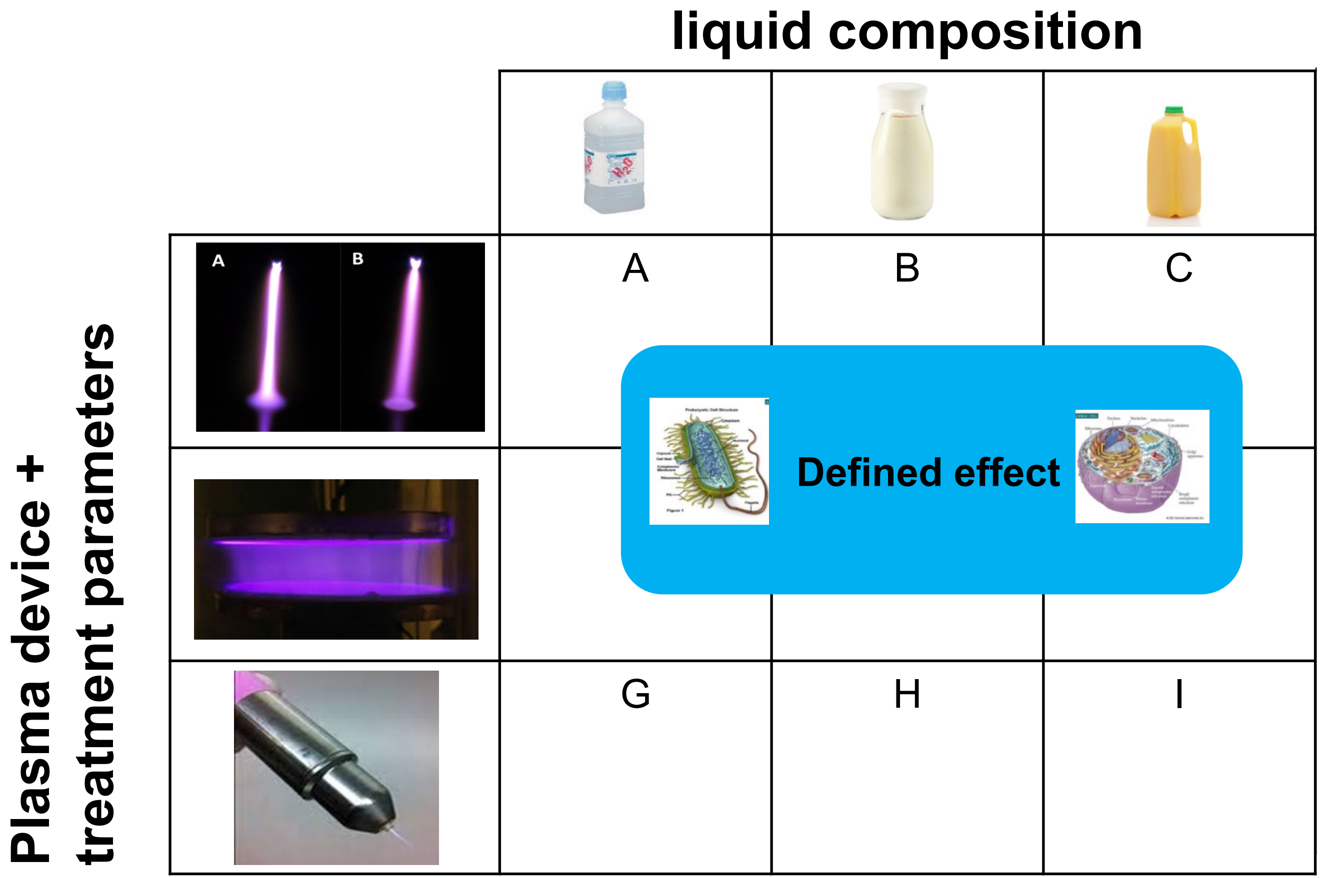
Conclusion

- **PFL can be**
  - Storable (limited shelf-life at RT, extended shelf-life in frozen state)
  - Controllable (chemistry - device, discharge, liquid parameters)
  - Stable (temperature)
  - Modifiable? (influencing secondary reactions)
- **Antimicrobial efficacy depends on**
  - Concentration and type of ROS/RNS
  - Low pH
  - Contact time
  - Microbial species

Outlook - Challenges and opportunities

- **<u>Engineering</u>**
  - Selectivity
  - Scalability (Process assurance, reproducibility)
  - Storability
- **<u>(Bio)Chemistry</u>**
  - Reactive species
  - Molecular modifications
  - Biochemical/cellular mechanisms
- **<u>Application</u>**
  - Versatility
  - Mode of application
    - Washing
    - Vapourization/fumigation
    - freezing

Tailoring plasma functionalized liquids for specific applications?



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Thank you!

Questions?

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