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Plasma-Liquid Chemistry in the Presence of Organic Matter

Katharina Stapelmann
Assistant Professor
Department of Nuclear Engineering, North Carolina State University

U.S. DEPARTMENT OF ENERGY
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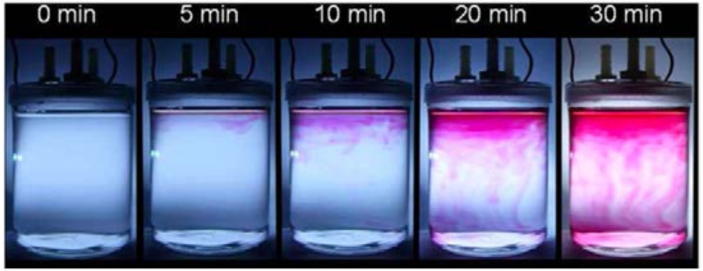
Overview

- Plasma-Liquid Chemistry for Bio Applications
- Organic matter in liquids becomes part of the chemistry
  - "Passive": offering a target for reactions
  - "Active": cells actively change chemistry

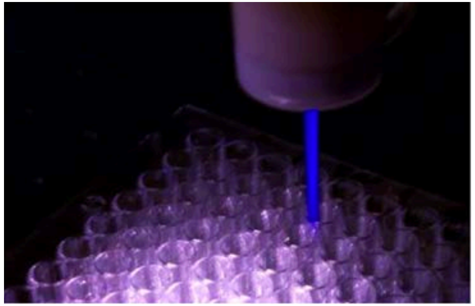


https://doi.org/10.1088/0963-0252/25/5/053002

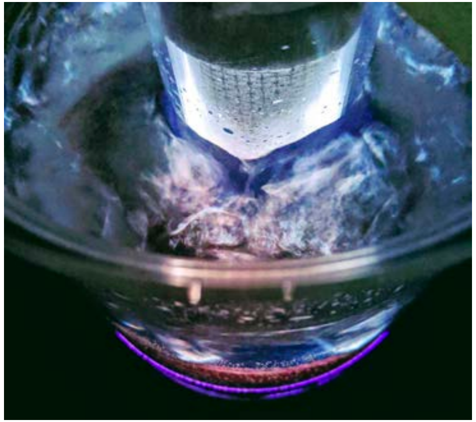
Motivation

- Plasma Medicine: cell culture treatment
  - Cell medium different reactions than in water
  - How do cells impact the liquid chemistry?
- Plasma Agriculture: treatment of water
  - How clean is the water?
  - Transition to real-life applications

How does the chemistry change when organic matter is present?



Plasma **2018**, *1*(1), 47-60



www.plasmaleap.com

COST Reference Microplasma Jet

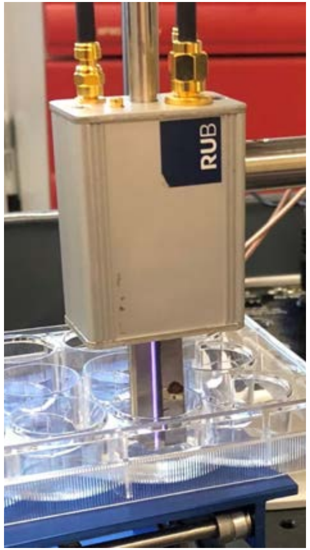
COST Reference Microplasma Jet

- Designed as reference source: robust and reproducible
- Allows to contextualize results
- CCP, 13.56 MHz RF, He, He/H_2O and He/O_2
- 1 mm electrode distance, 30 mm plasma channel
- Integrated matching network along with current and voltage probes for continuous monitoring

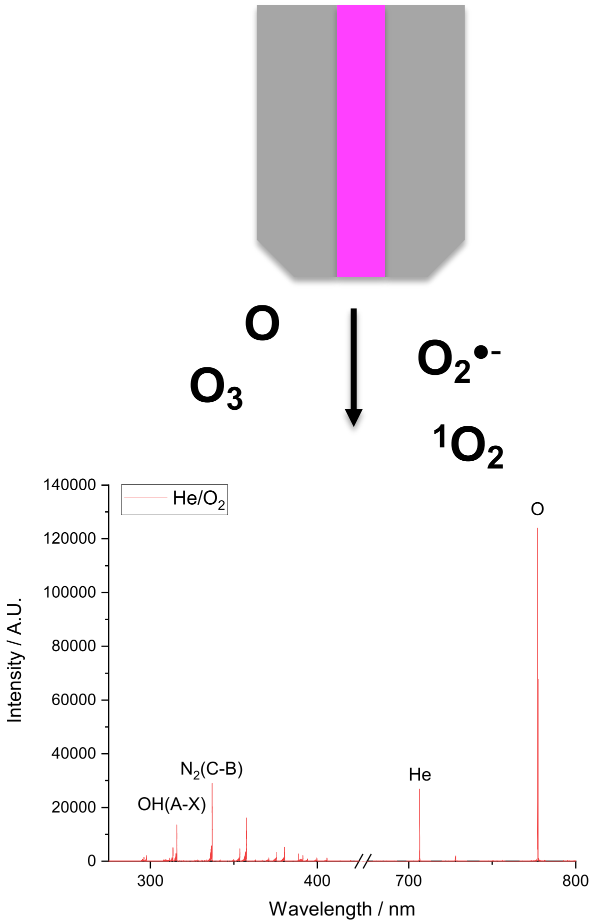
Golda J et al. Journal of Physics D: Applied Physics. 2016;49(8):84003.

Experimental Conditions

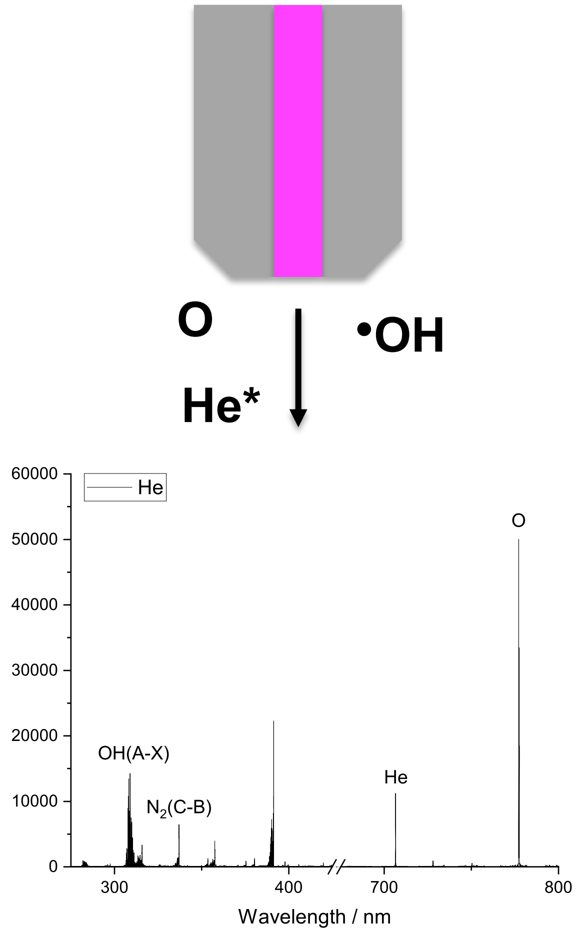
- Total gas flow: 1 slm
- Power constant at 750 mW
  - ~240 V_{RMS} for He/O_2 and He/H_2O
  - ~215 V_{RMS} for He
- Liquid treatments:
  - 12-well plate, 1 ml treatment volume
  - 4 mm distance from nozzle to liquid
- All measurements performed in triplicate



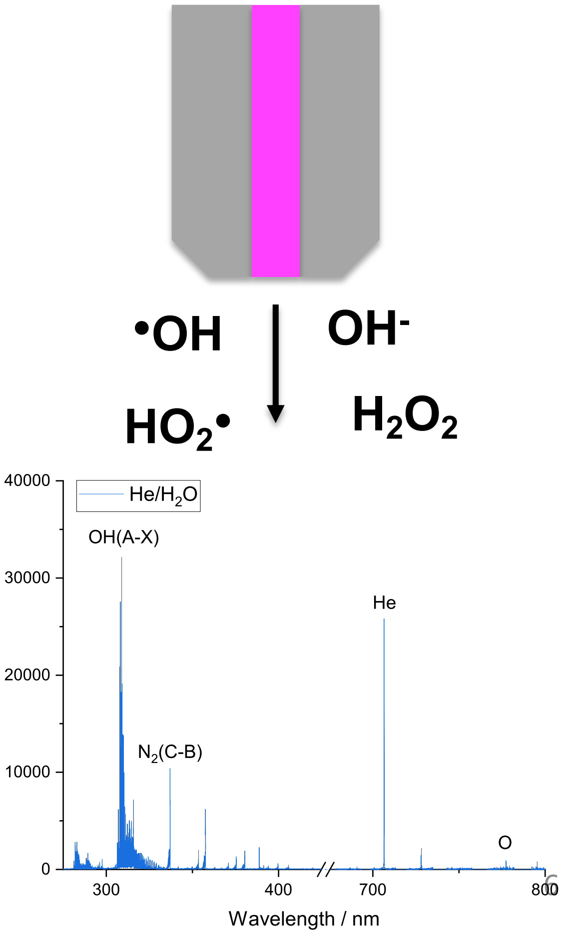
He 0.6% O_2



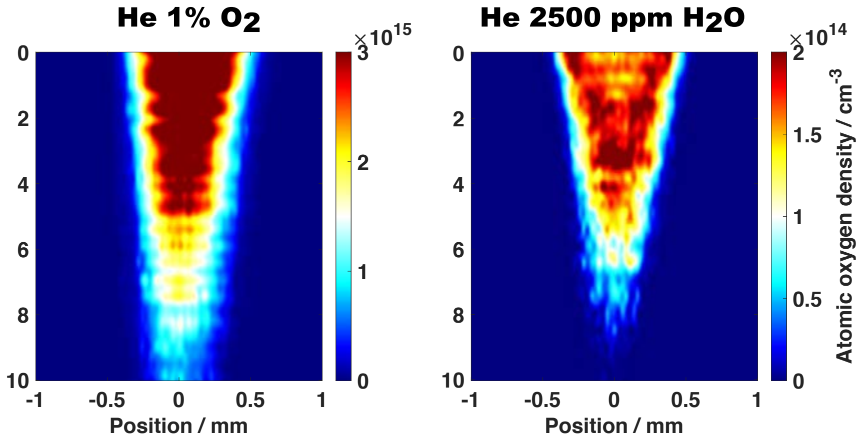
He-only

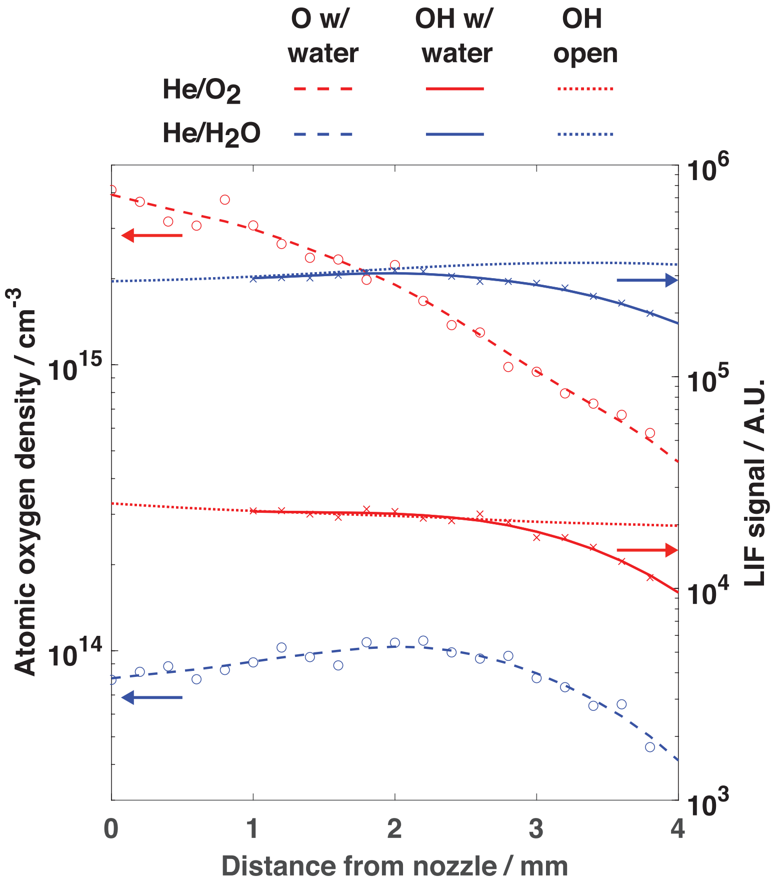


He 2500 ppm H_2O



O and OH in He/O_2 and He/H_2O

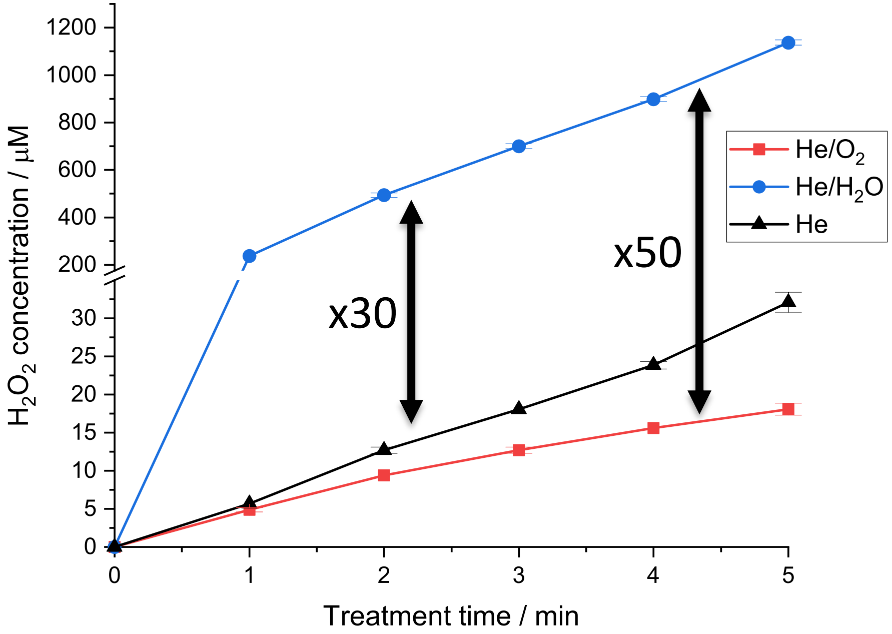




- O (TALIF) and OH (LIF) in an open effluent and with a liquid interface present at 4 mm distance
- O dominates in He/O_2, OH in He/H_2O

Myers, B. et al. *Journal of Physics D: Applied Physics* **54** (2021): 145202
Stapelmann, K. et al. *Journal of Physics D: Applied Physics* **54** (2021): 434003

Start simple: H_2O_2 formation in DI water

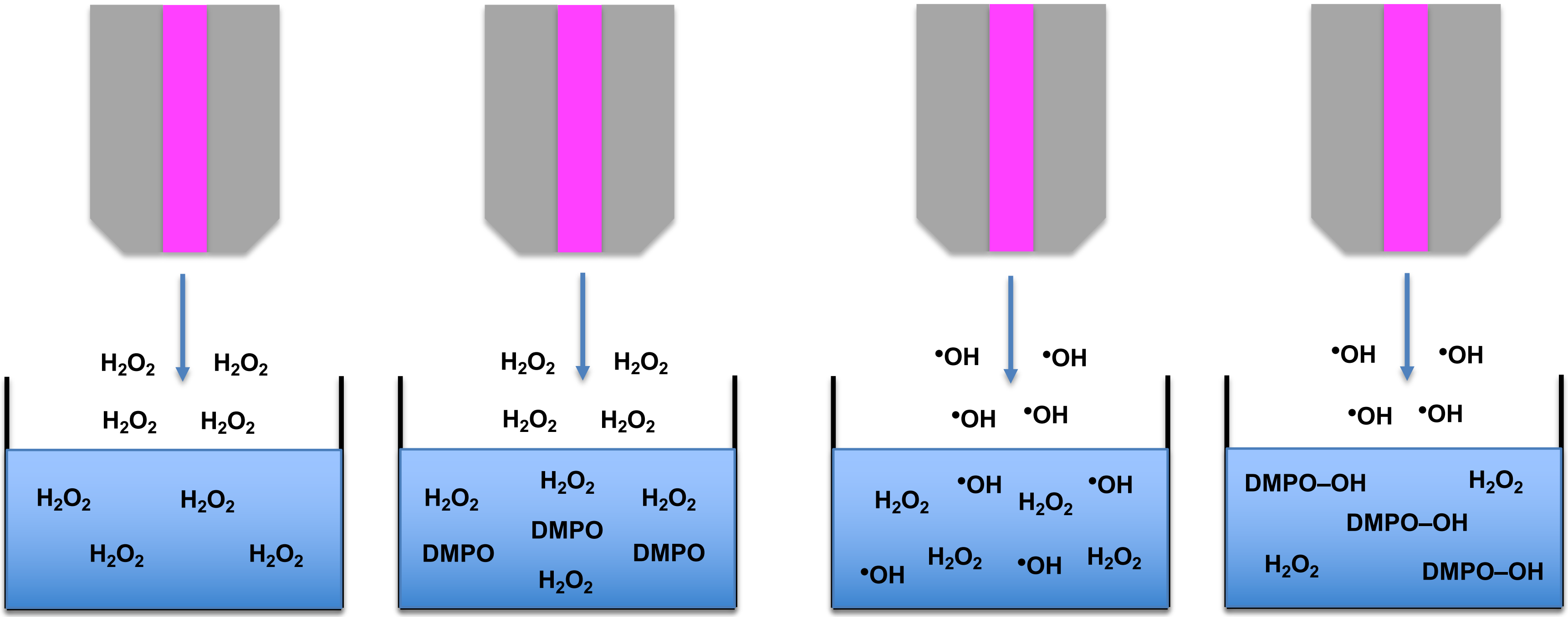


- H_2O_2 in He/H_2O plasma-treated sample is ~30x higher than He, ~50x higher than He/O_2
- Corresponds well to OES and previously reported gas phase measurements*

*Benedikt J et al. *Plasma Sources Sci Technol*. 2016;25(4):045013.

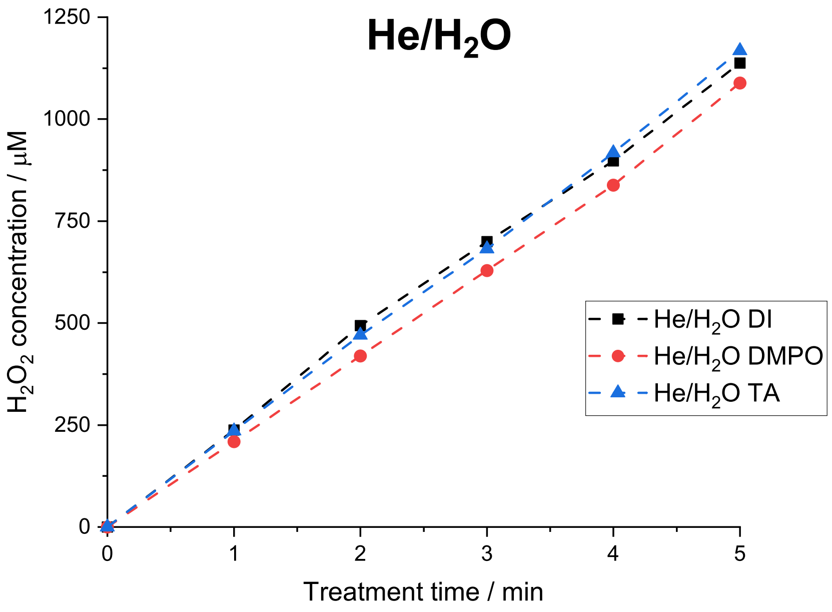
Isolating H_2O_2 origins using OH scavenger

H_2O_2 is produced primarily in gas phase | **H_2O_2 is produced primarily in aqueous phase**



DI water | 50 mM DMPO | DI water | 50 mM DMPO

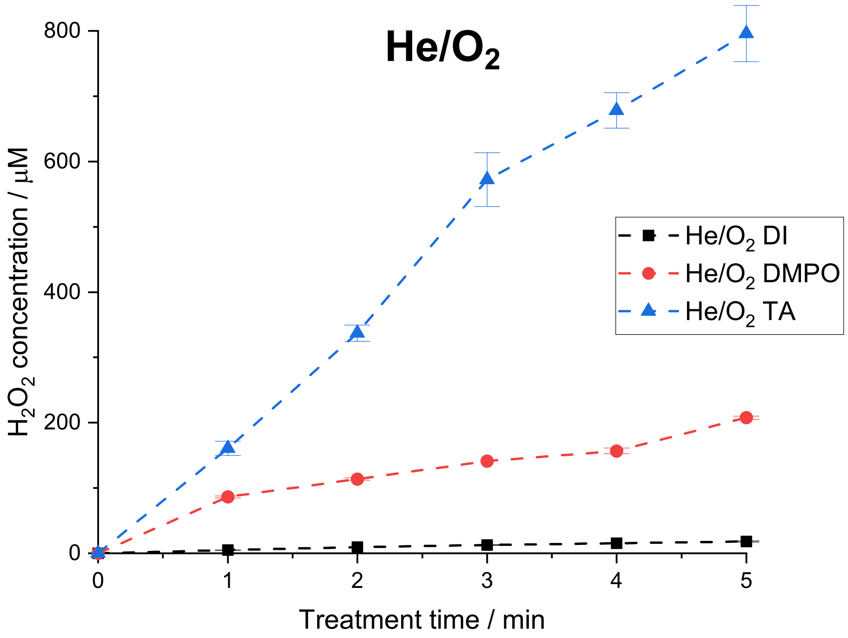
Isolating H_2O_2 origins using OH scavenger



- OH scavengers: Terephtalic acid (TA) and spin-trap 5,5-Dimethyl-1-pyrroline N-oxide (DMPO)
- H_2O_2 ~ constant across solutions
  - $^\bullet OH + {}^\bullet OH \rightarrow H_2O_2$ ($k = 5 \times 10^9\ M^{-1}s^{-1}$)
  - $DMPO + {}^\bullet OH \rightarrow DMPO\text{–}OH$ ($k = 4.3 \times 10^9\ M^{-1}s^{-1}$)
- H_2O_2 must be produced exclusively in the gas phase

Myers, B. et al. *Journal of Physics D: Applied Physics 54.14 (2021): 145202*

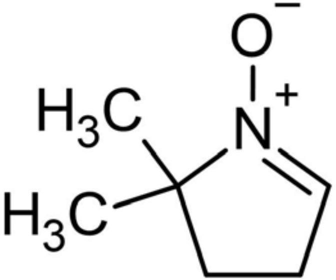
Isolating H_2O_2 origins using OH scavenger



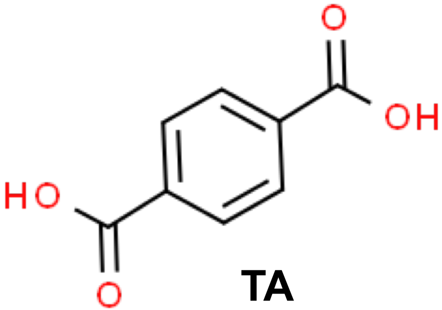
- H_2O_2 varies significantly between solutions
- Observed previously in phenol*
  - H abstraction by O from C-H bonds
- O enters liquid and reacts further to form H_2O_2
  - OH or HO_2 as precursor?

Myers, B. et al. *J. Phys. D: Appl. Phys.* 54.14 (2021): 145202 *Hefny, M. et al. *J. Phys. D: Appl. Phys.* 2016;49(40):404002.

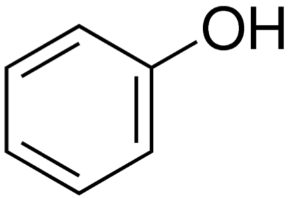
Comparison DMPO, TA, Phenol



DMPO



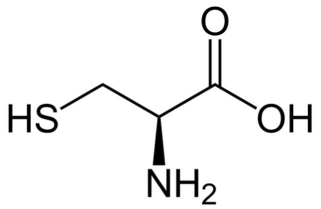
TA



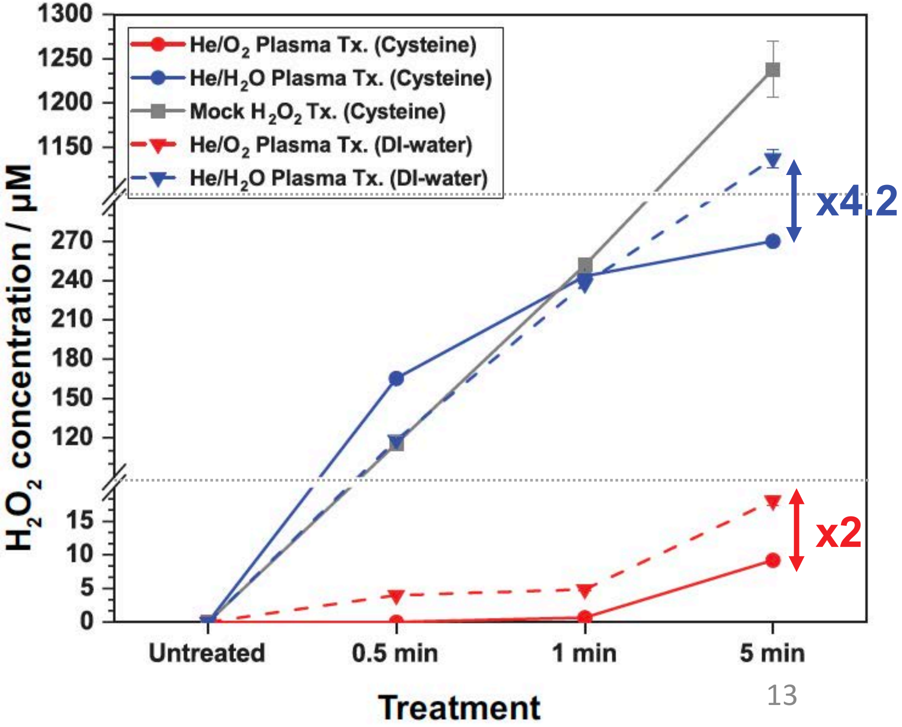
Phenol

- Ring structures
  - H abstraction by O from C-H bonds
- O enters liquid and reacts further to form H_2O_2
- When ring structures are present, H_2O_2 production increases – ring structures become part of the liquid chemistry

From Ring Structures to Cysteine



- Amino acid cysteine as simple model
- He/H_2O
  - up to 1 min.: DI ~ cys ~ cys + H_2O_2
  - 5 min: mock > DI >> cys
  - Cys consumed H_2O_2
  - Not if only H_2O_2 is present: short-lived species necessary to initiate reactions
- He/O_2
  - DI water higher H_2O_2 concentration
  - Cys consumed H_2O_2



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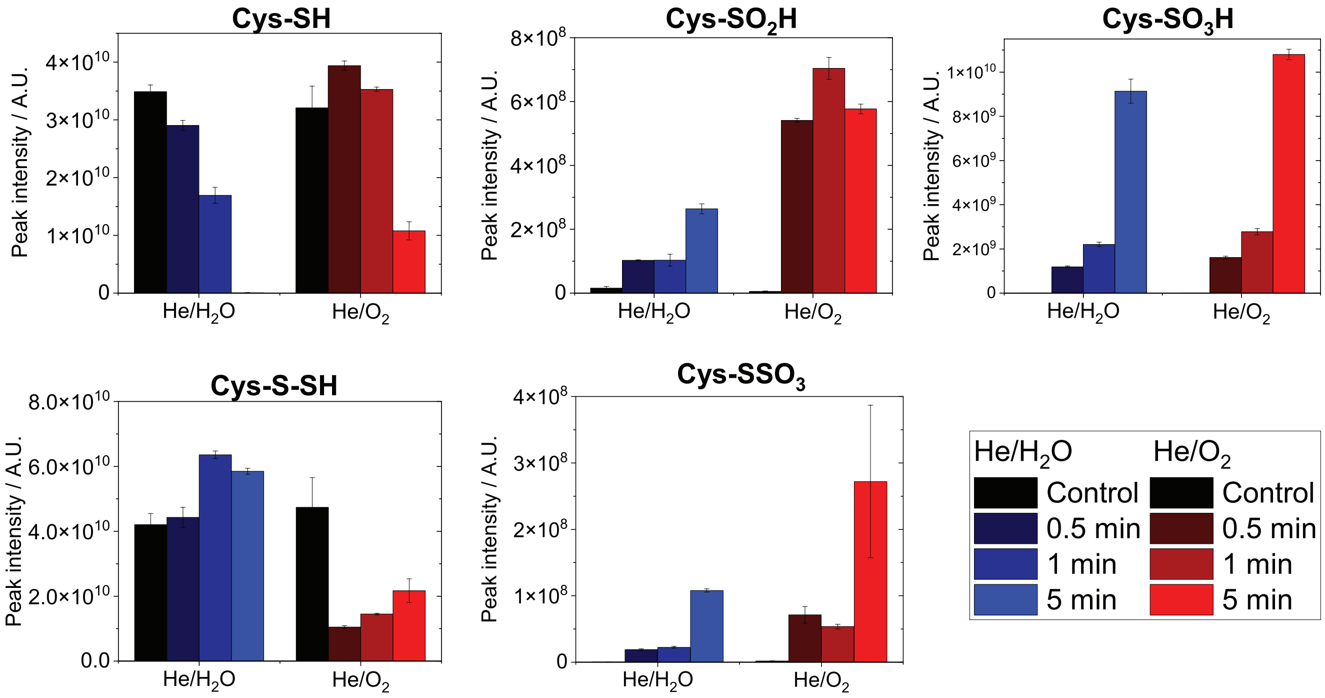
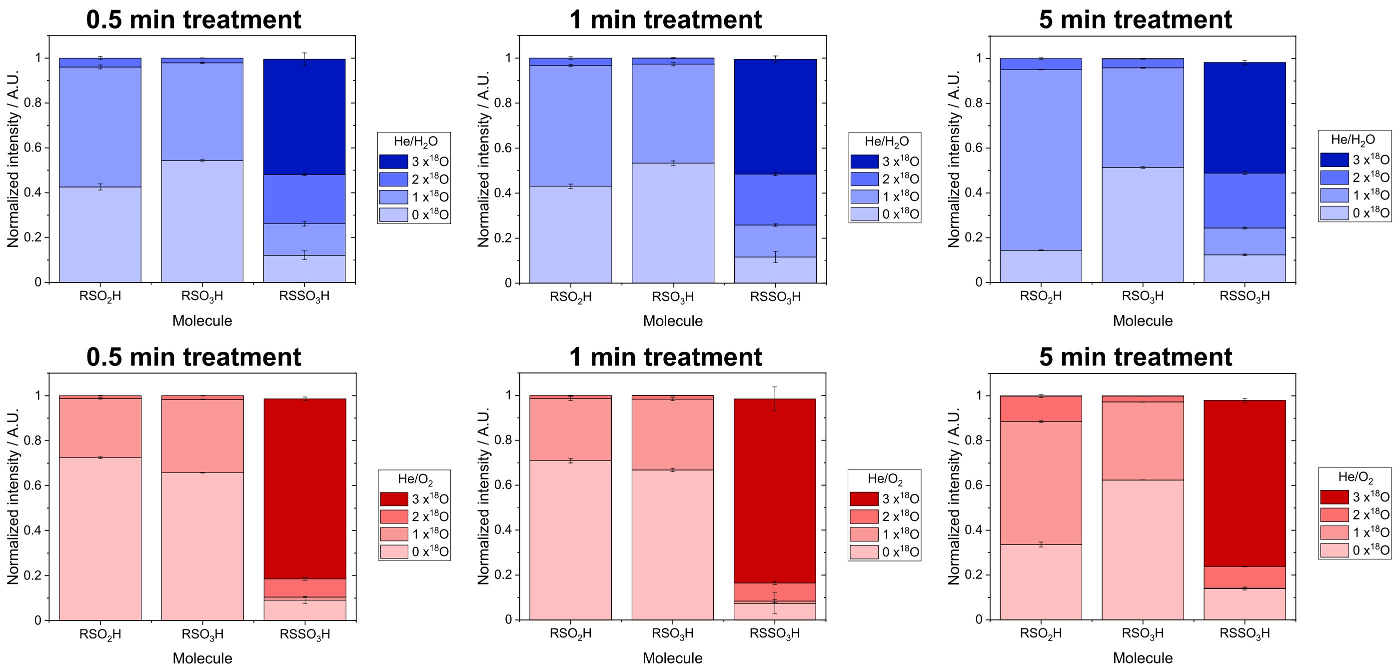
Cysteine Modifications



- Native cysteine disappears completely after 5 min He/H_2O
- Primary modification He/H_2O: cystine and variations
- Primary modification He/O_2: oxidation of sulfur

Stapelmann, K. et al. *Journal of Physics D: Applied Physics* **54** (2021): 434003

Cysteine Modifications – Origin of Species



- Heavy water $H_2{}^{18}O$ as liquid
- He/H_2O: more ^{18}O incorporated
- ^{18}O due to hydrolysis, (V)UV or metastable impact, or ^{16}OH H hopping

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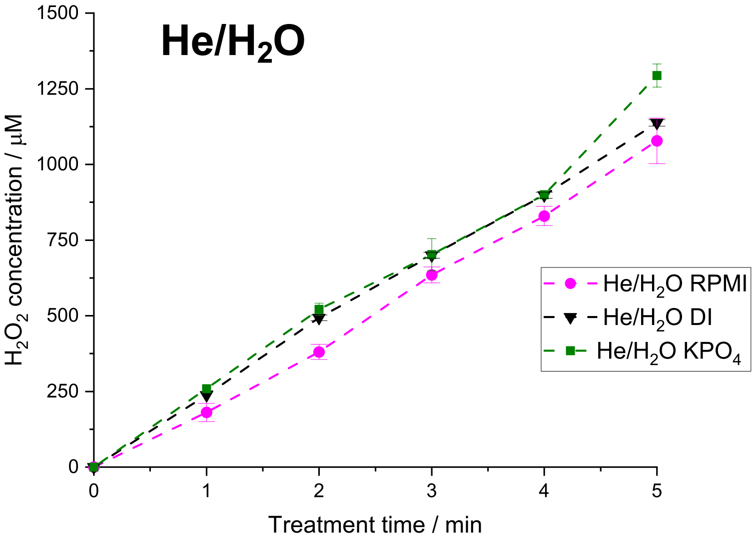
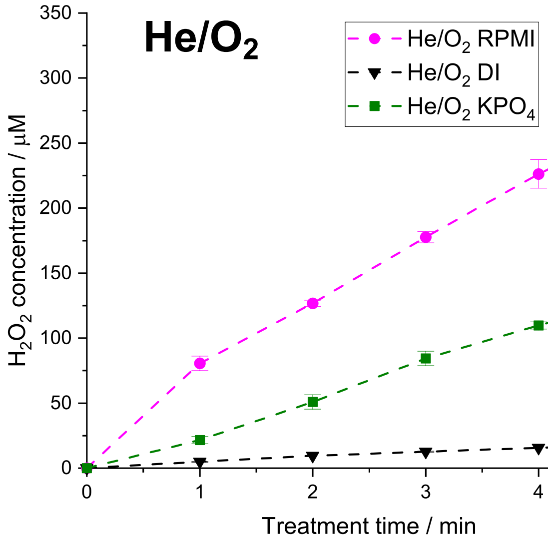
Increasing complexity – buffer & medium





- Cell culture medium (RPMI) and buffer (KPO_4) used for plasma medicine experiments influence H_2O_2 production for He/O_2
- Likely due to O interacting with organics in the medium

Closer look

RPMI Medium

Glycine	L-Isoleucine	L-Tryptophan
L-Arginine	L-Leucine	L-Tyrosine
L-Asparagine	L-Lysine	L-Valine
L-Aspartic Acid	L-Methionine	
L-Cystine	L-Phenylalanine	Vitamins
L-Glutamine	L-Proline	Inorganic Salts
L-Histidine	L-Serine	Glucose
L-Hydroxyproline	L-Threonine	Glutathione

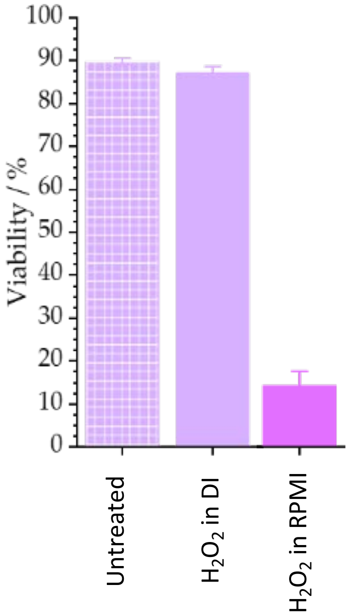
RPMI cell medium: a lot of components!

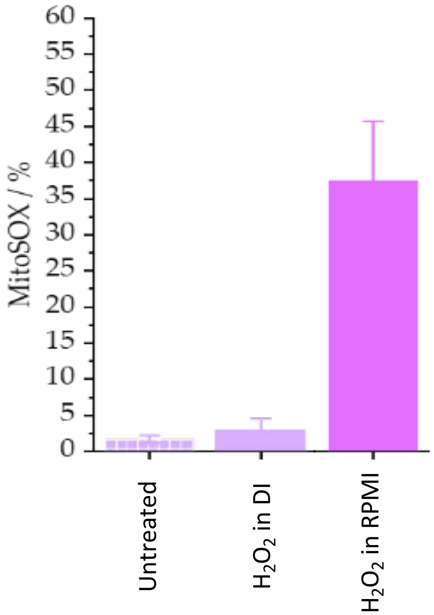
- 3 amino acids with ring structures
- 2 sulfur-containing amino acids

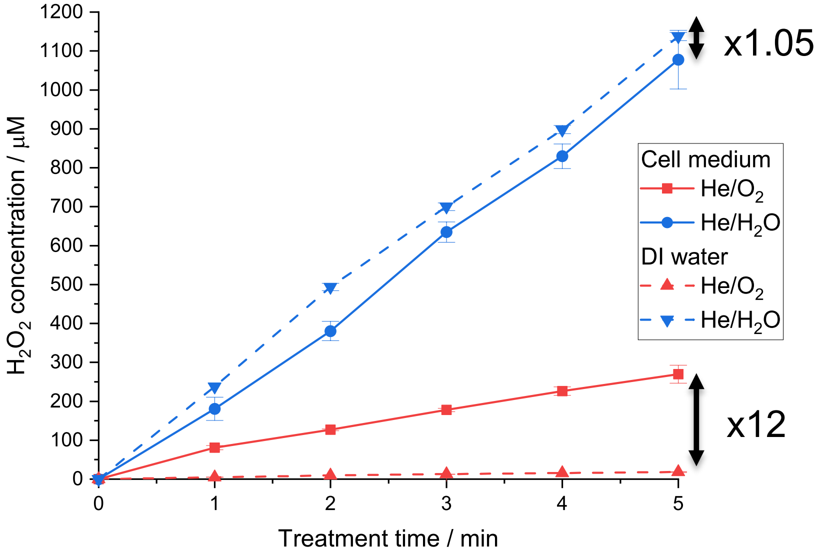
What does it mean for plasma medicine?

Mock treatments to isolate effects – measure species in correct solution

He/O_2 based mock treatment of Jurkat cells



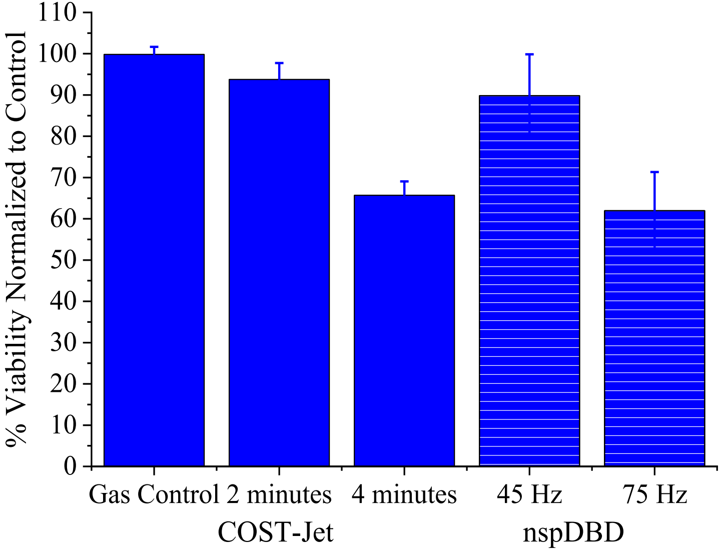




Ranieri P et al. *Applied Sciences*. 2020;10(6):2025.

What happens when cells are present?

- Intercomparison study COST jet and nsp-DBD
- Completely different setups, different chemistry, …
- Cell viability as starting point
  - Jurkat cells ($3 \cdot 10^4$ cells per well)
  - Incubated for 24 hr prior to assesing cell viability
  - Image cytometry
- Conditions with comparable cell viabilities:
  - COST jet 2 min ~ nspDBD 45 Hz (10 s)
  - COST jet 4 min ~ nspDBD 75 Hz (10 s)



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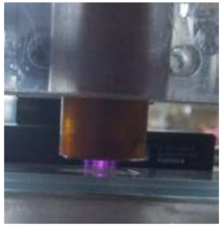
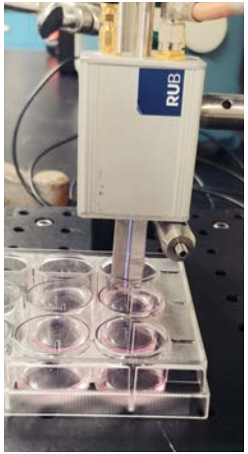
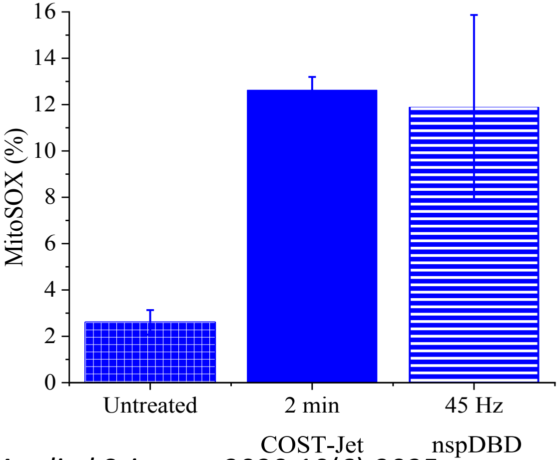


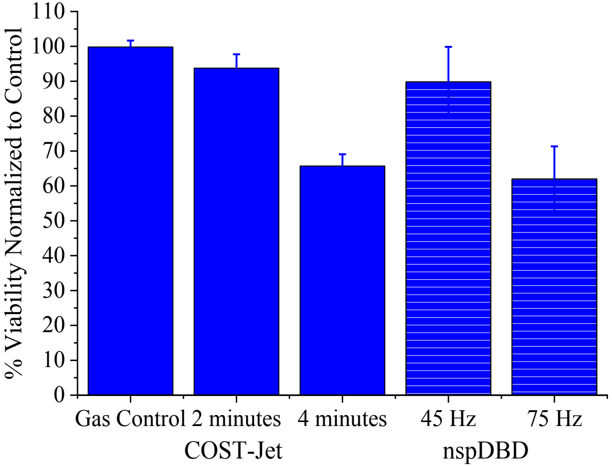
nspDBD and COST jet
- Cell produced chemistry



Not only the cell viability is comparable, but also the mitochondrial superoxide production in response to plasma treatment

COST jet 2 min ~ nspDBD 45 Hz (10 s)





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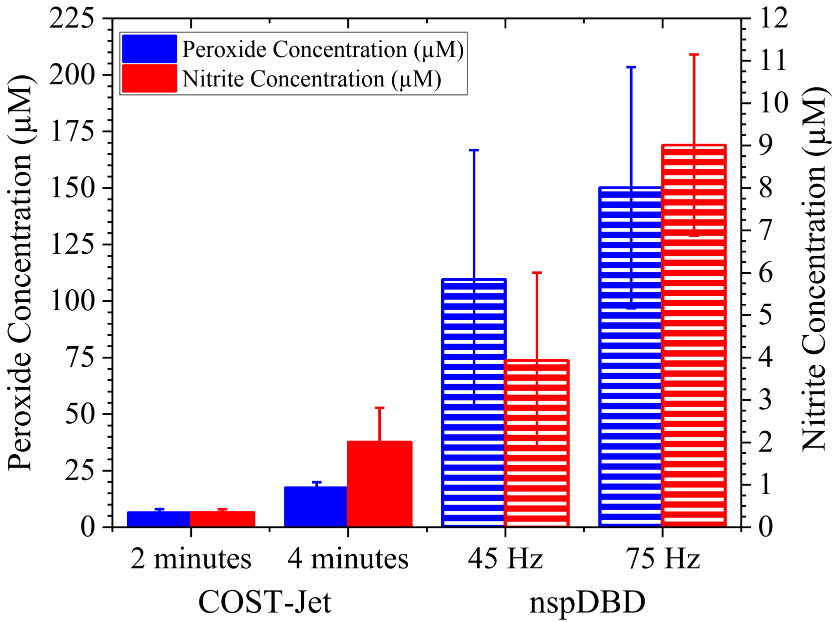
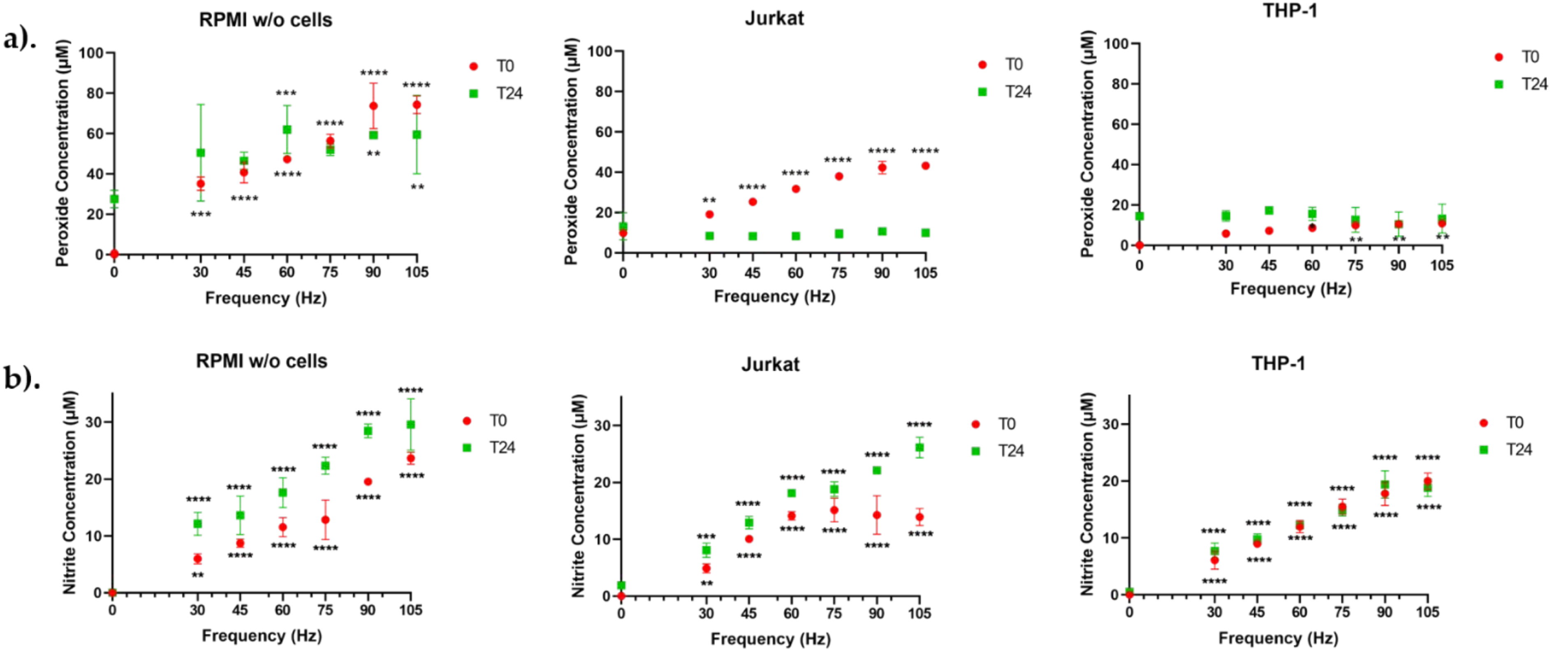
COST jet and nspDBD
- Liquid chemistry



- As expected, the liquid chemistry is completely different
- Long-lived species such as H_2O_2 and nitrite **not** (exclusively) responsible for cell death

Ranieri P et al. *Applied Sciences*. 2020;10(6):2025.

nspDBD - Liquid chemistry with cells



H. Mohamed et al. *Cancers*. 2021;13(10):2437.

Conclusions

- Organic matter becomes part of the chemistry
- Different types of organic matter affect chemistry differently
  - By offering a target for reactive species / precursors for other long-lived species (e.g. OH / H_2O_2)
  - By providing new precursors to form other species (e.g. H + HO_2 / H_2O_2)
- Living cells actively contribute to liquid chemistry
  - By offering a target for reactive species
  - By uptake and neutralization of ROS
  - By releasing reactive species (TBD)

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