Biomedical Plasmas:

plasma oncology and more

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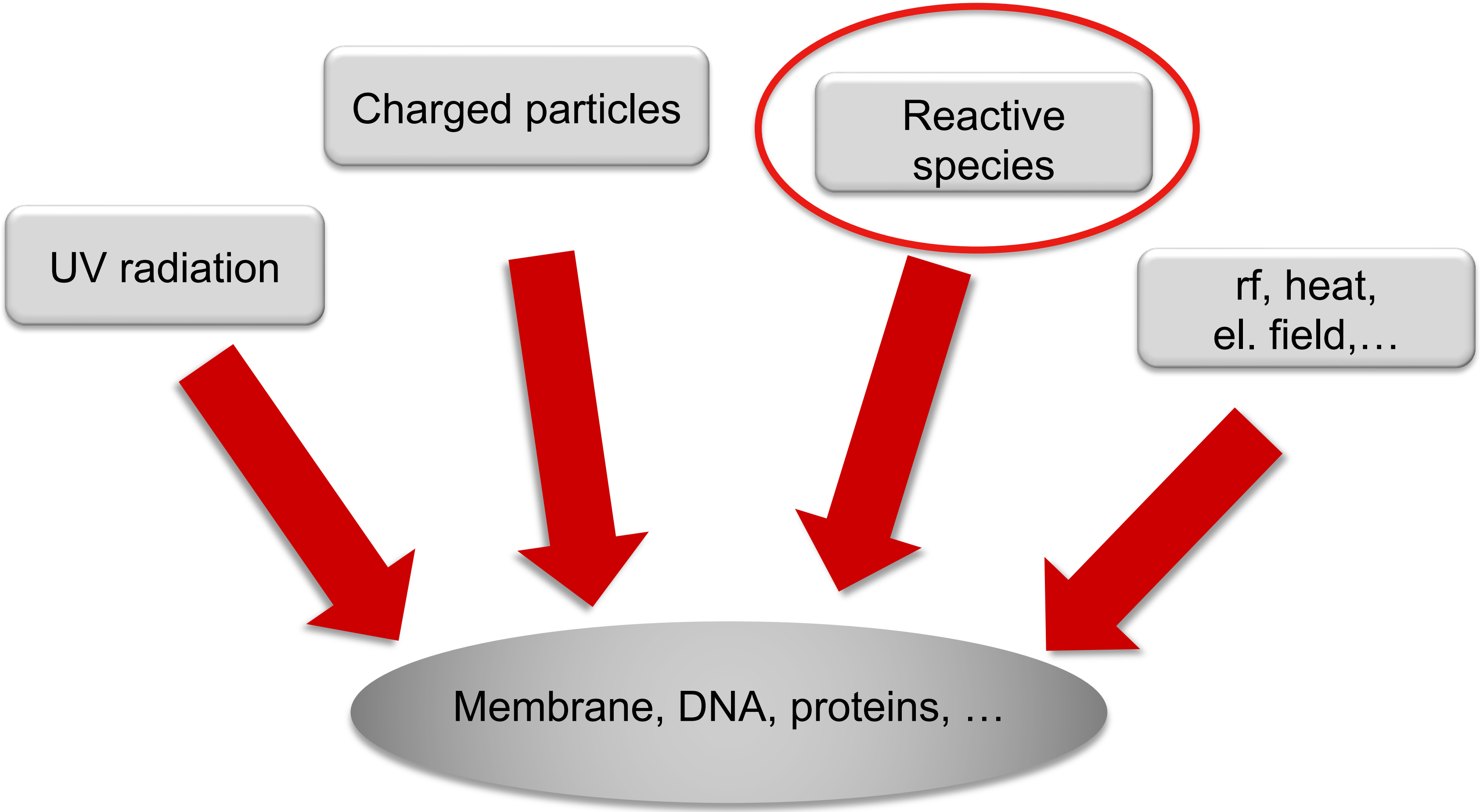
Overview

- Non-thermal plasma and its active components
- What is cancer?
- How does plasma oncology work?

- Plasma-liquid interactions in the presence of cells
- Plasma for Life Sciences – Research Overview
- Outlook

Plasma – a cocktail of active ingredients



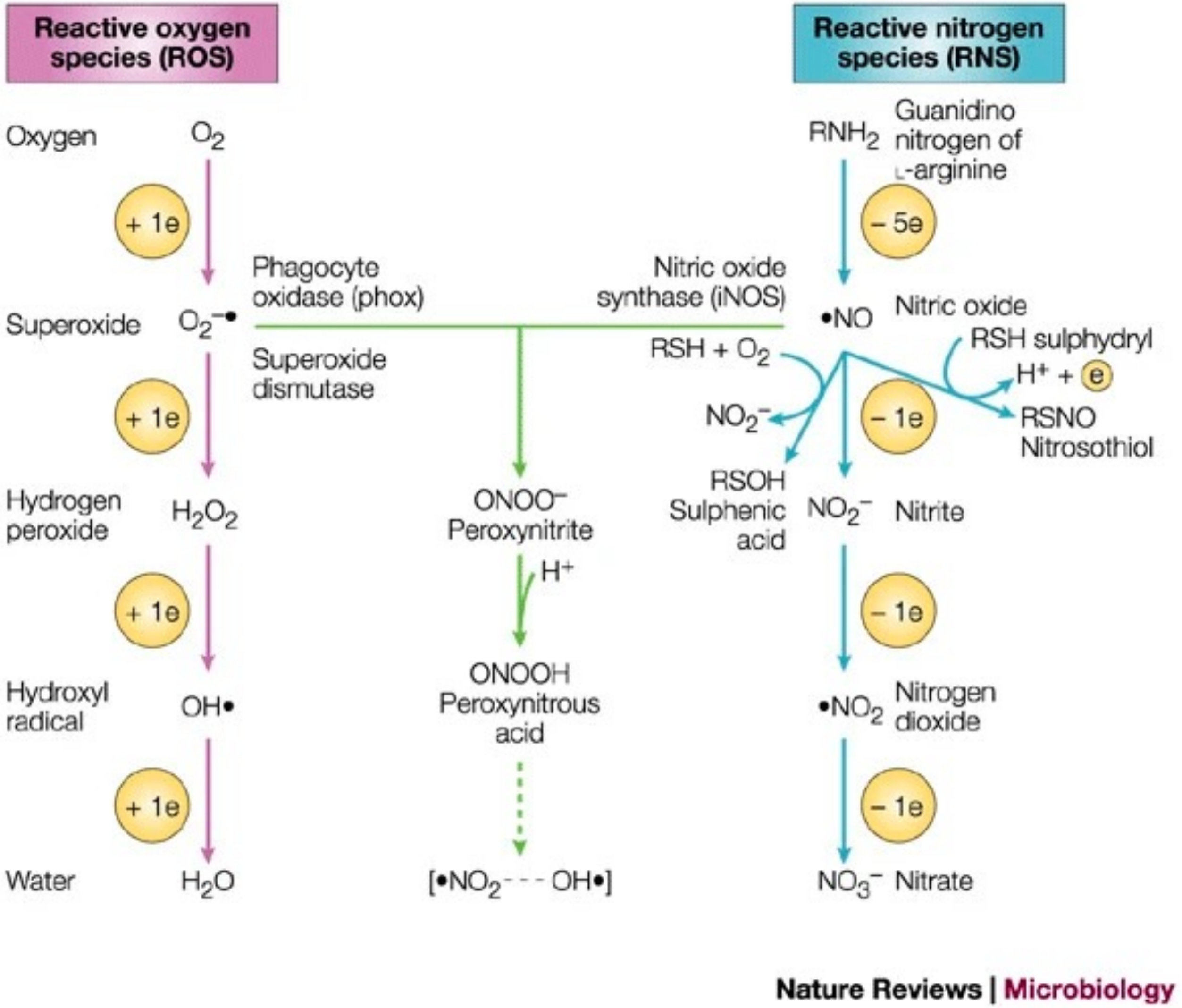
Key Roles of Reactive Oxygen and Nitrogen Species

- Plasma (in air) produces the same molecules that our body uses for signaling
- RONS generally react by exchanging electrons in a chemical process called redox reactions (reduction-oxidation)

> Looking at life from the perspective of electron flow may be one of the most universal and fundamental approaches to Biology. This is because all known life forms depend on electrons that get stranded at the top of 'energy hills,' waiting to roll down the hill toward a low-energy resting place. This insight has been famously expressed in the words of Albert Szent-Gyorgyi: "Life is nothing but electrons looking for a place to rest" [2].

Comprehensive Clinical Plasma Medicine, Springer 2018

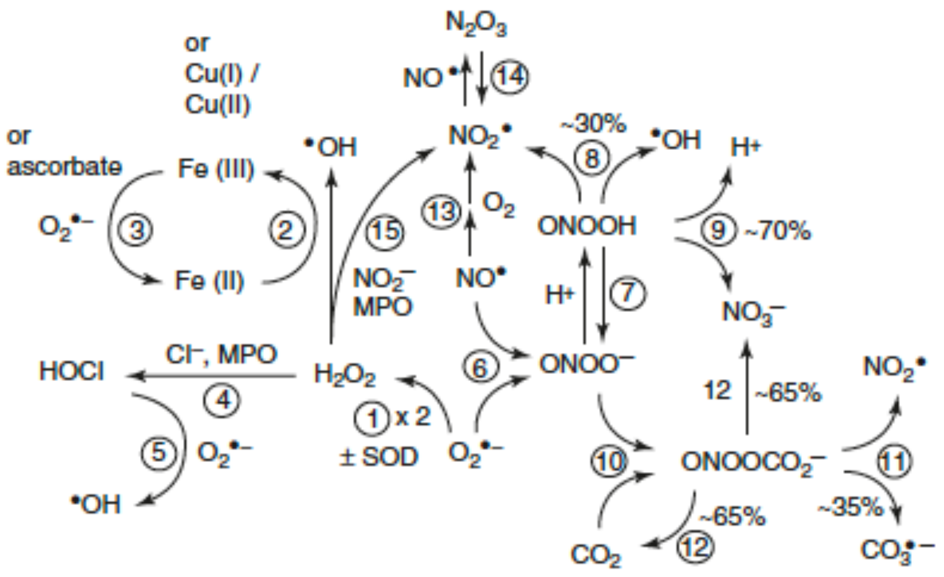
RONS & Redox



Fang, F. Antimicrobial reactive oxygen and nitrogen species: concepts and controversies. *Nat Rev Microbiol* **2,** 820–832 (2004). LINK

Redox Biology – NO and O_2^-

- NO and O_2^- together with other RONS have short lifetimes. If applied on the surface, any effect that occurs deeper in the tissue must come from the biological response of the system ("biological penetration depth" vs "physical penetration depth")
- NO and O_2^- are created in biological systems and by plasma!



Comprehensive Clinical Plasma Medicine, Springer 2018

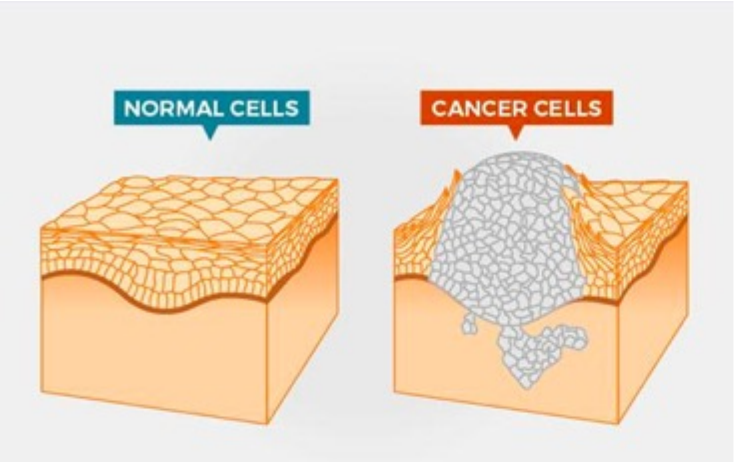
Redox Biology

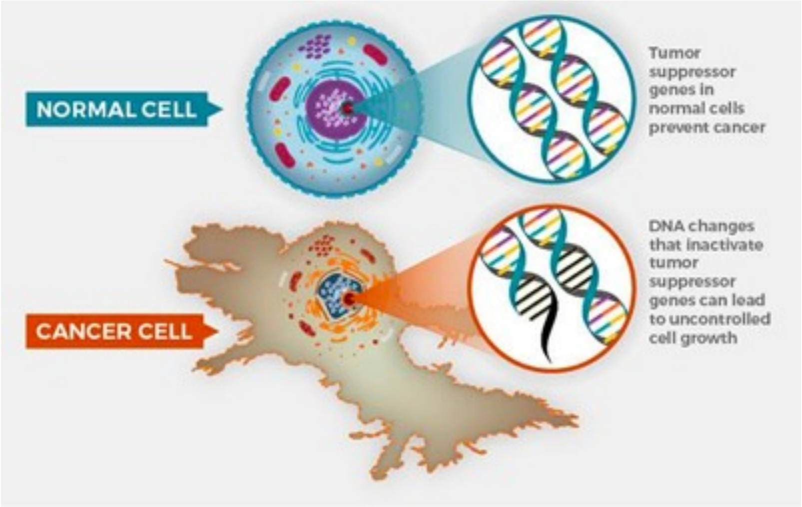
- RONS-based therapies & plasma cause similar effect as an innate immune system oxidative burst
- Plasma (at low doses) mimics an immune response to tissue damage, wounds or infection which could initiate a natural healing response
- Plasma & Immune Response – Immunotherapy for Cancer Treatment?

Comprehensive Clinical Plasma Medicine, Springer 2018

Cancer – what is it exactly?

- malignant growth resulting from the division of abnormal cells
- Cancer is caused by changes in the DNA
- A DNA change can cause genes involved in normal cell growth to become oncogenes
    - Unlike normal genes, oncogenes cannot be turned off: uncontrolled cell growth
- Tumor suppressor genes prevent cancer in normal cells by stopping cell growth.
- DNA changes that inactivate tumor suppressor genes can lead to uncontrolled cell growth.

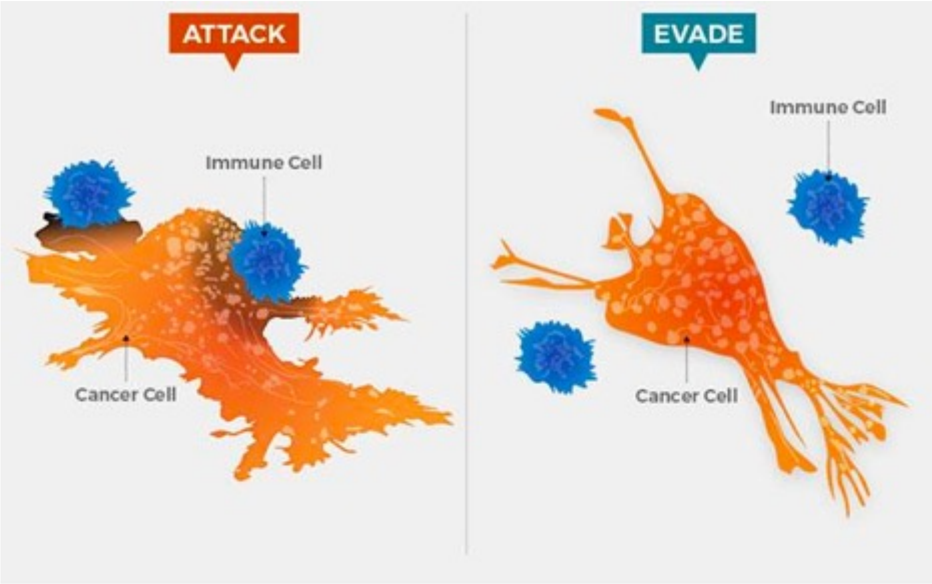




www.cancer.gov

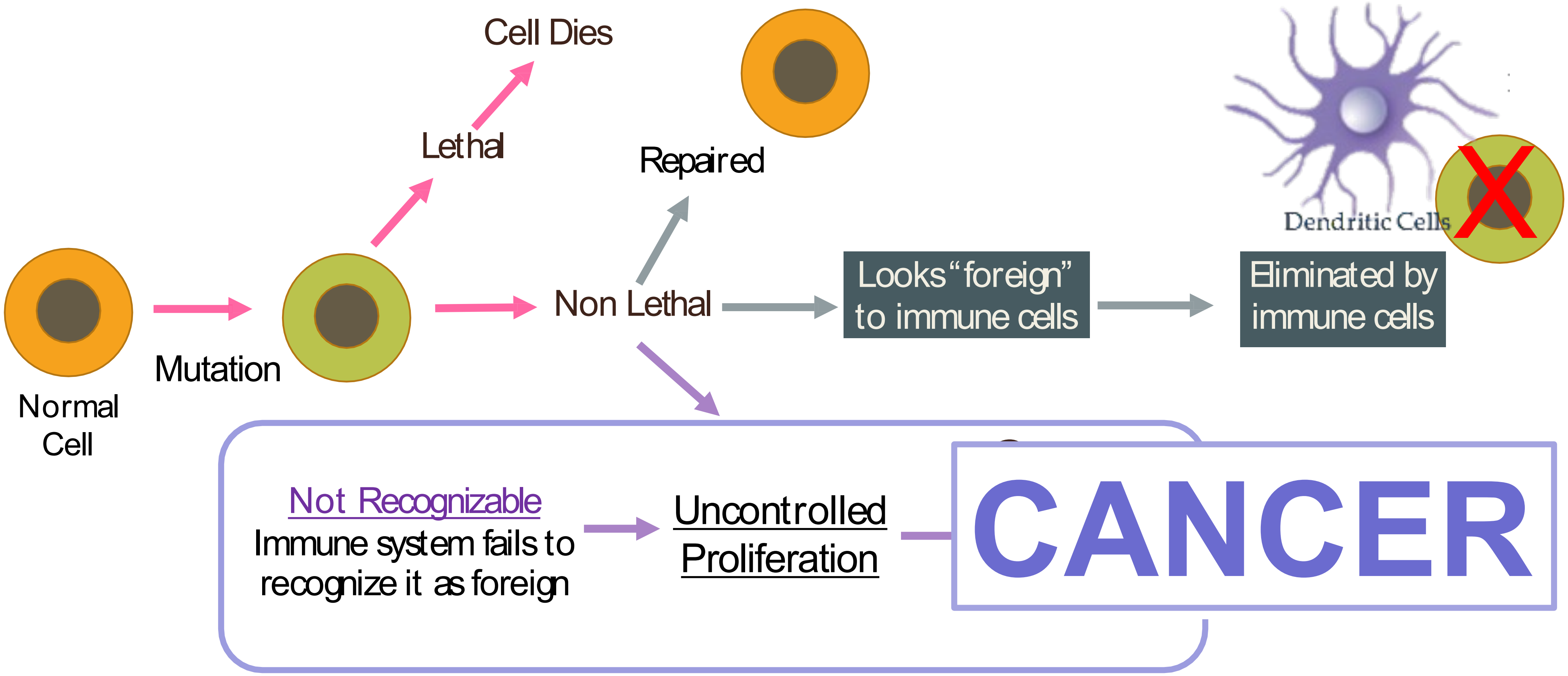
Cancer – Immune Escape

- Immune Escape:
  - Cancer cells can be detected and attacked by the immune system
  - Some cancer cells can avoid detection or thwart an attack
  - Immune therapy / plasma can help the immune system to detect and kill cancer cells

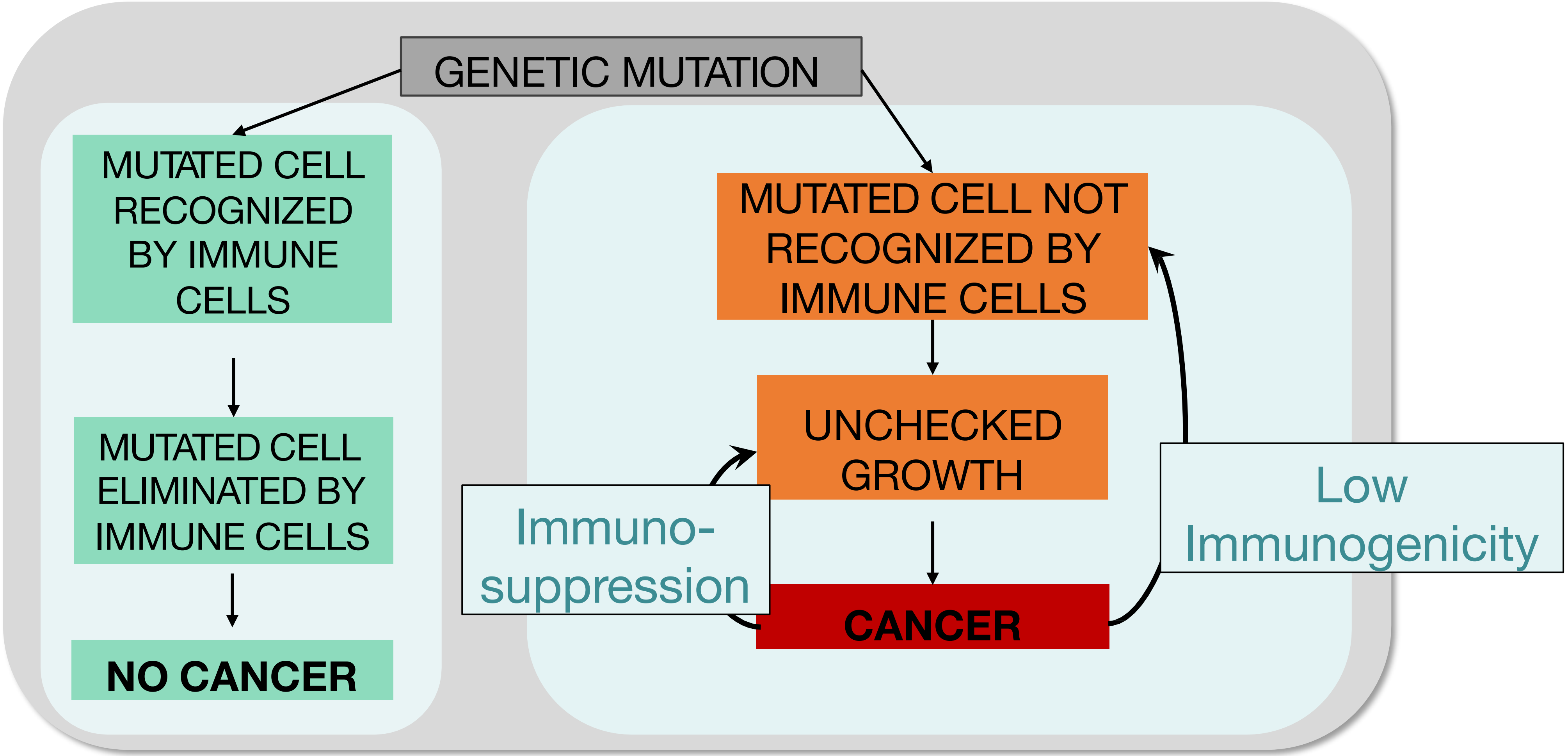


www.cancer.gov

ROLE OF IMMUNE SYSTEM IN CANCER



ROLE OF IMMUNE SYSTEM IN CANCER



TREATMENT OF CANCER

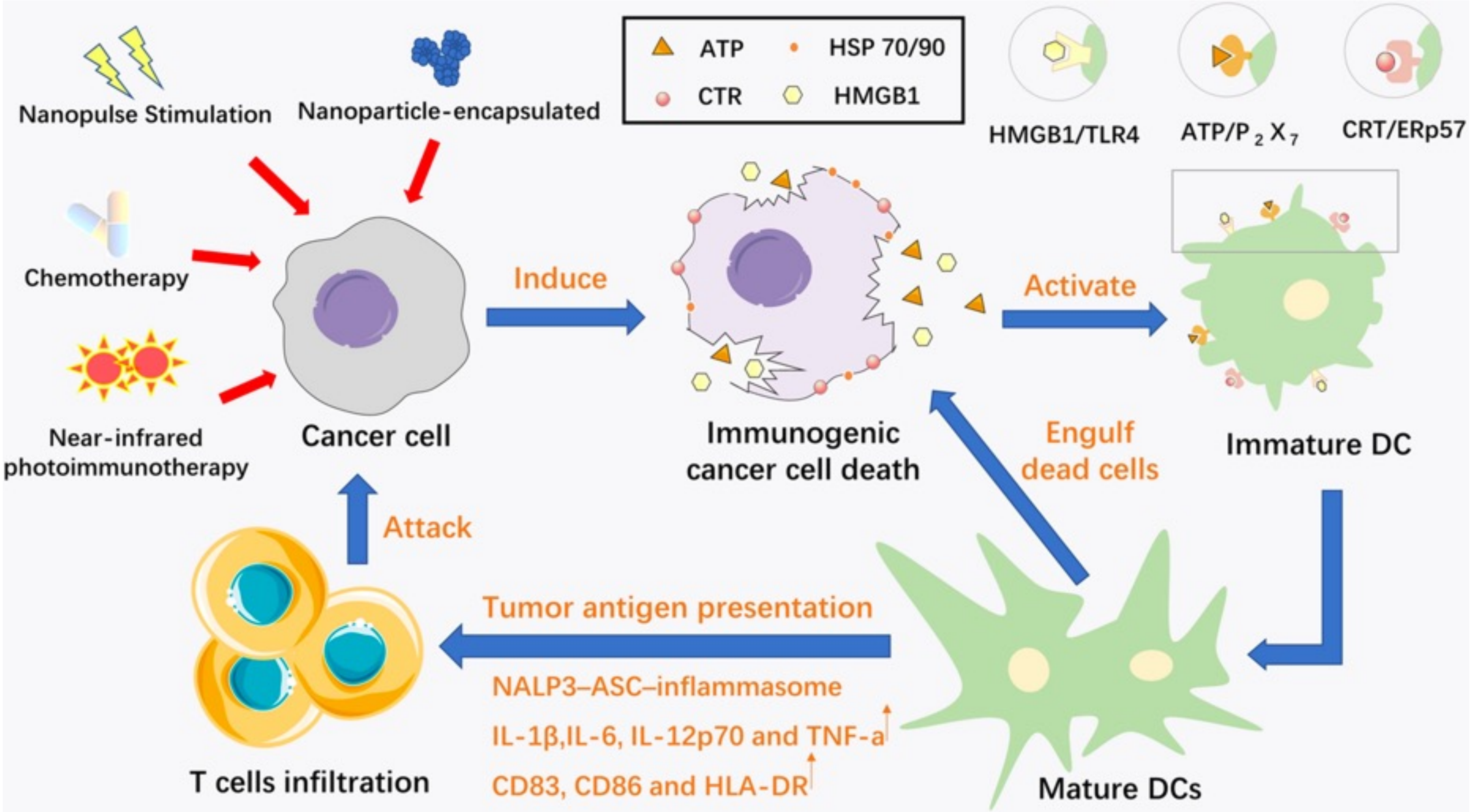
- Goal: get rid of cancer cells - immune benefit incidental
  - ★ Surgery
  - ★ Chemotherapy
  - ★ Radiation Therapy
  - ★ Photodynamic Therapy
- Immunotherapy: targeted delivery of immune “attack”
  - ★ Engineered immune cells (CAR T cells)
  - ★ Overcoming immune inhibition (immune checkpoint blockage)
  - ★ **IMMUNOGENIC CELL DEATH (ICD) induction**

ADVANTAGES OF IMMUNOTHERAPY

- Durable - immune cells remember tumor cells and prevent recurrence. Cure?
- Targeted - little to no damage to normal tissue
- Adaptable - as tumors change, immune responses evolve
- Synergistic - can complement and build on other cancer therapies
- Systemic - can target and destroy tumor cells anywhere in the body

Immunogenic cell death in cancer therapy

+ Plasma?

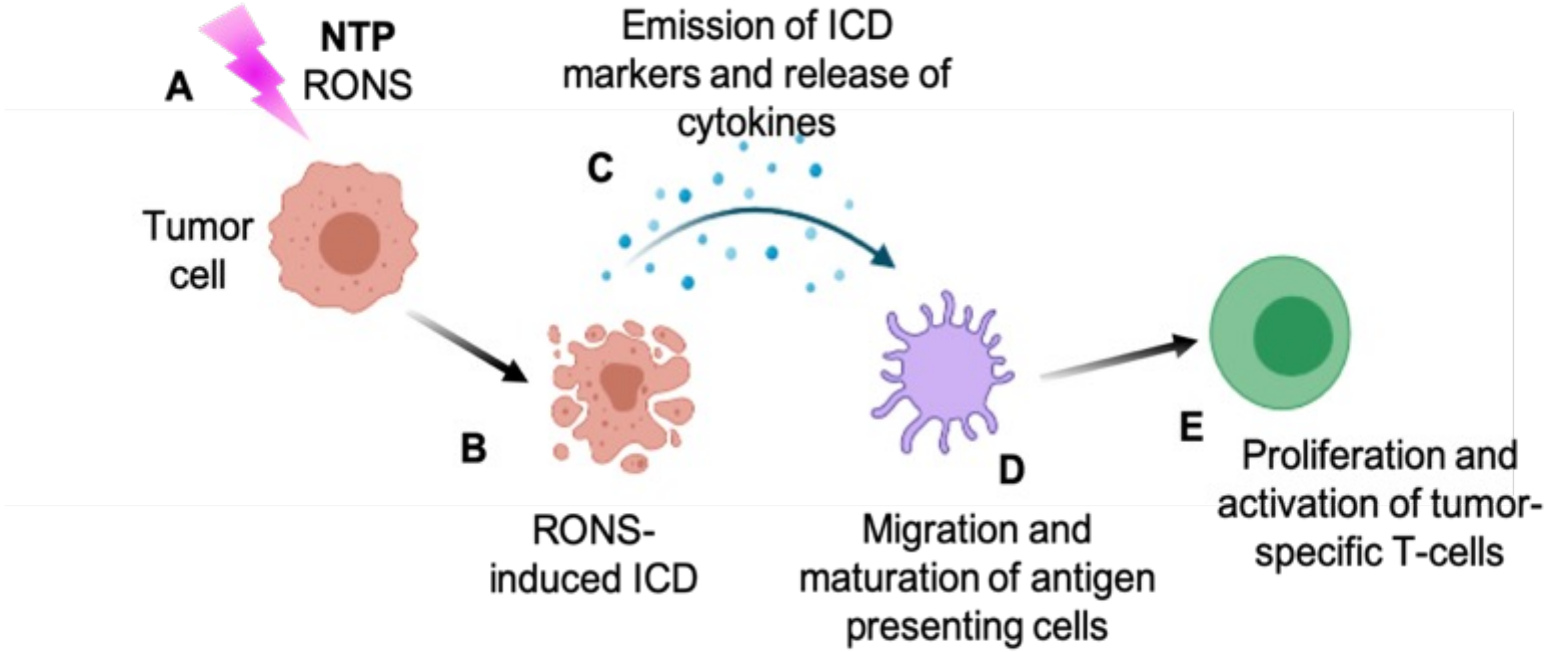


https://doi.org/10.1111/jcmm.14356

RONS and immunogenic cell death

- Tumors employ **immunosuppressive strategies** to escape the body's normal immune surveillance and elimination
- Exposure of antigens on tumor cells via the **immunogenic cell death**
- Several steps in this pathway are **ROS dependent**
- High ROS amount leads to **cell death and debulking of tumor mass**, the smaller tumor may be more manageable by the compromised immune system
- Removal of immunosuppressive cells by plasma can be an additional beneficial outcome

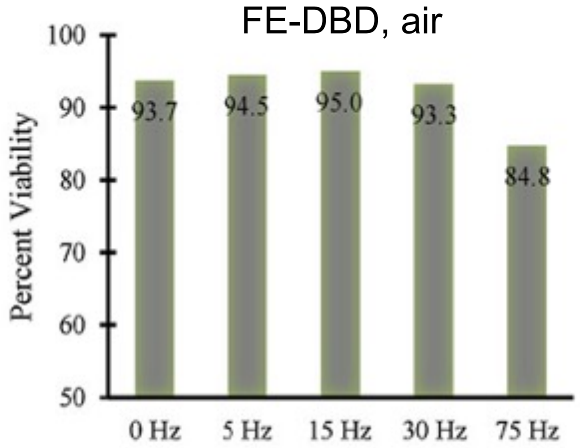
Conceptual Hypothesis

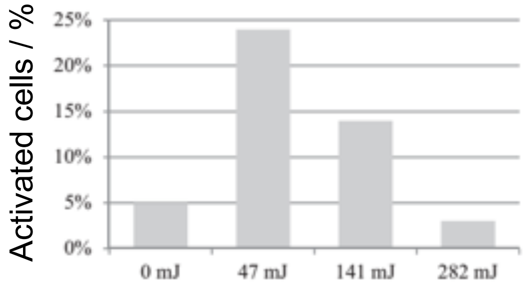


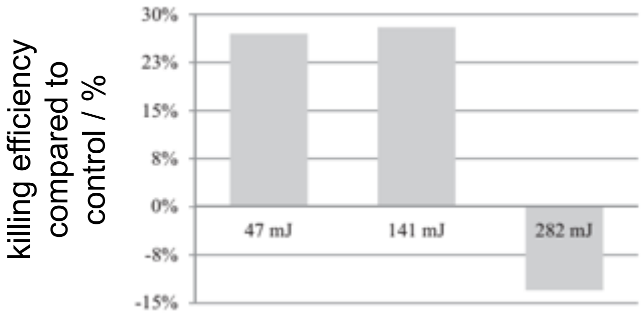
Exposure of tumor cells to NTP-produced RONS (A) leads to RONS-induced immunogenic cell death (ICD) (B) followed by emission of ICD-related markers and the release of key pro-inflammatory cytokines (C) that enhance migration and maturation of antigen presenting cells (D). These in turn stimulate the proliferation and activation of tumor-specific T-cells (E).

Immunotherapy with plasma – the early days

- "Immune Escape" big problem with cancer
- Idea: activate immune system with plasma
- Macrophages relatively insensitive against treatment





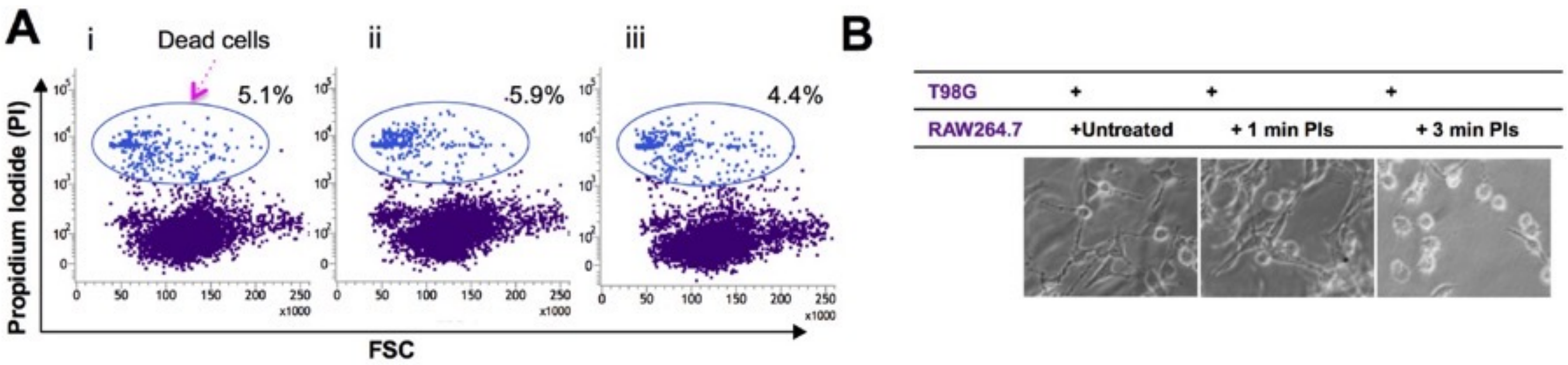


Miller et al. 2014 PPaP

- Activation dependent on treatment time
  - ROS activate macrophages but cause cell damage
- High energy activated macrophages
- But efficiency decreased
  - damaged cells less efficient
- Not the more the better!
- Regulation of immune system in small range

Macrophages induced cell death

- Plasma (DBD, air) activated macrophages can induce cancer cell death



- (A) Cell death of plasma treated macrophages after 24 h performed by propidium iodide (PI) staining analysis using flow cytometry. (i) 1 min plasma (ii) 3 min plasma (iii) control.
- (B) Morphology of glioblastoma co-cultured with macrophages and visualized by phase-contrast microscopy (Ti-U, Nikon) 48 h post-incubation.

Kaushik et al. 2016 JPhysD

Comparison of Two Cell Populations

- 2 leukemic cancer cell lines: Jurkat and THP-1
  - Jurkat cells are of lymphoid origin (precursors of T and B lymphocytes)
  - THP-1 cells are of myeloid origin (precursors of macrophages and dendritic cells), have the capacity to produce cellular RONS that are used for destroying pathogens
- Investigation of cell viability, damage-associated molecular patterns, phagocytosis by antigen presenting cells

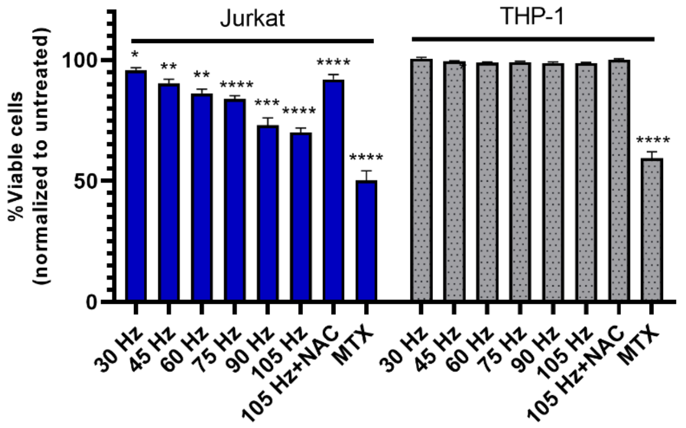
cancers MDPI

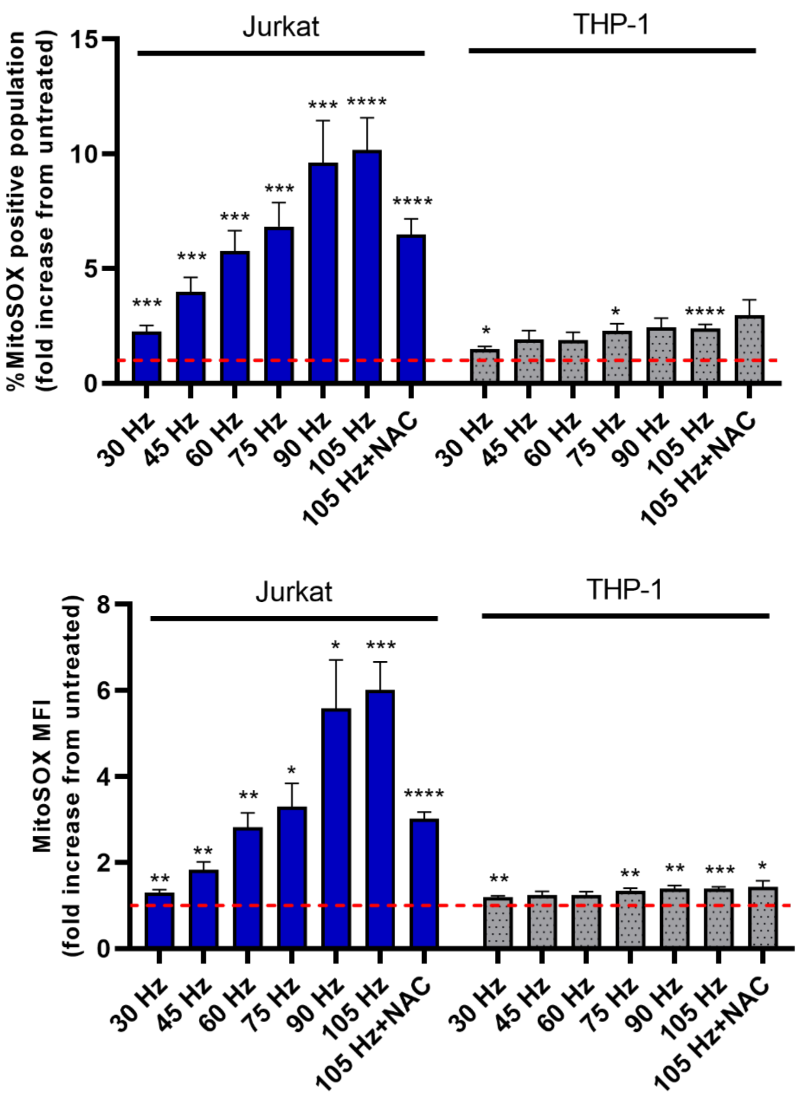
Article

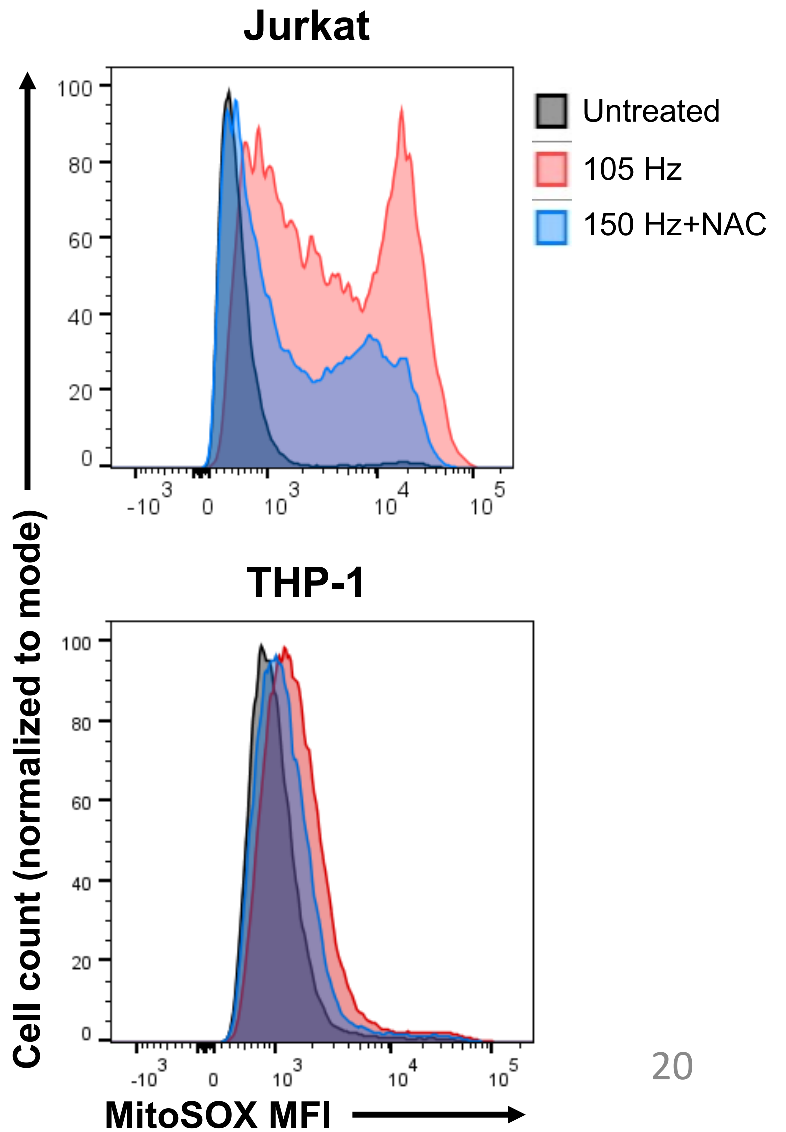
Differential Effect of Non-Thermal Plasma RONS on Two Human Leukemic Cell Populations

Hager Mohamed [1], Eric Gebski [2], Rufranshell Reyes [2], Samuel Beane [2], Brian Wigdahl [1], Fred C. Krebs [1], Katharina Stapelmann [3] and Vandana Miller [1,*]

Cell viability and MitoSox

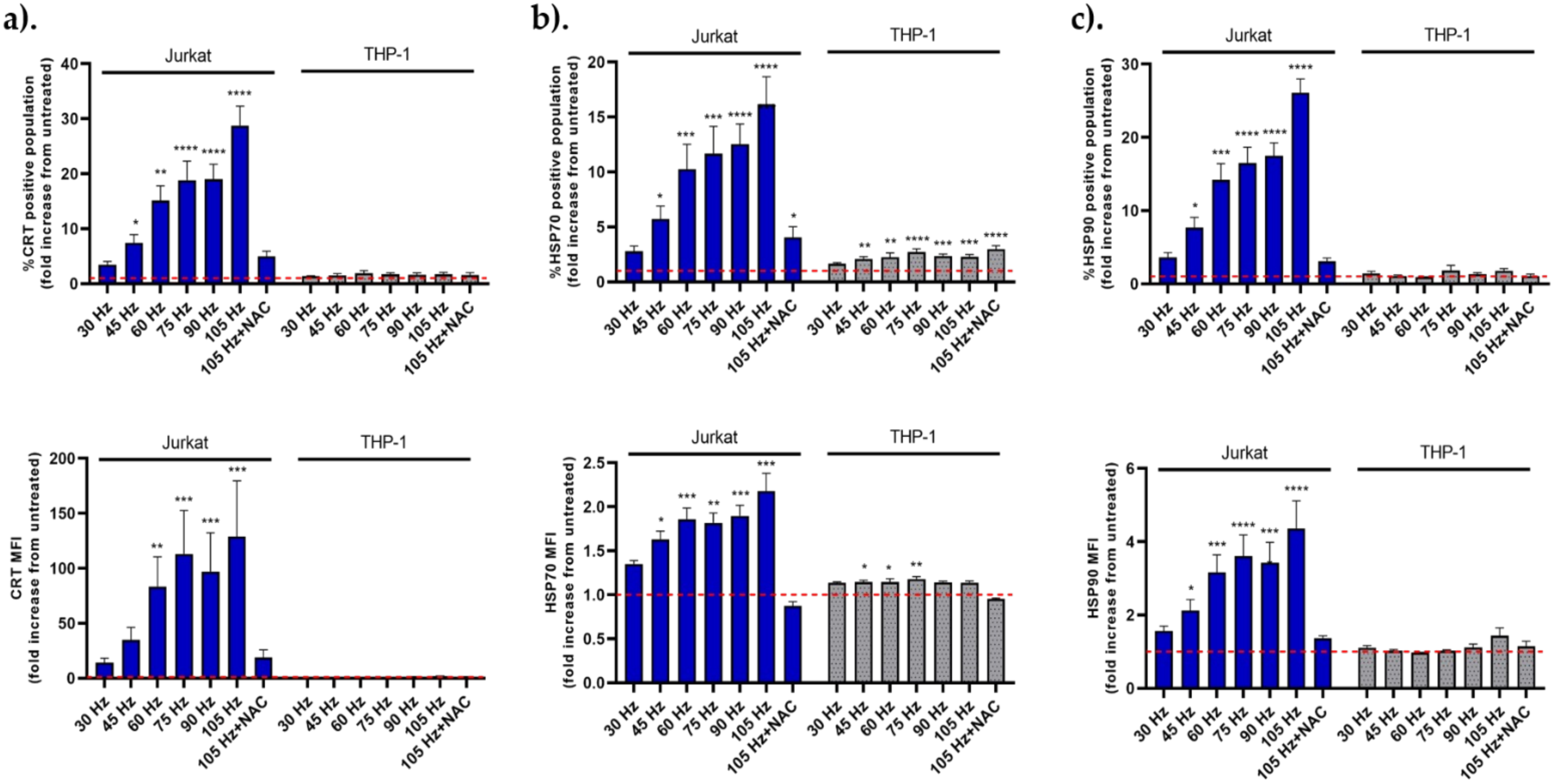






H. Mohamed et al. *Cancers*. 2021;13(10):2437.

Pro-phagocytic DAMPs



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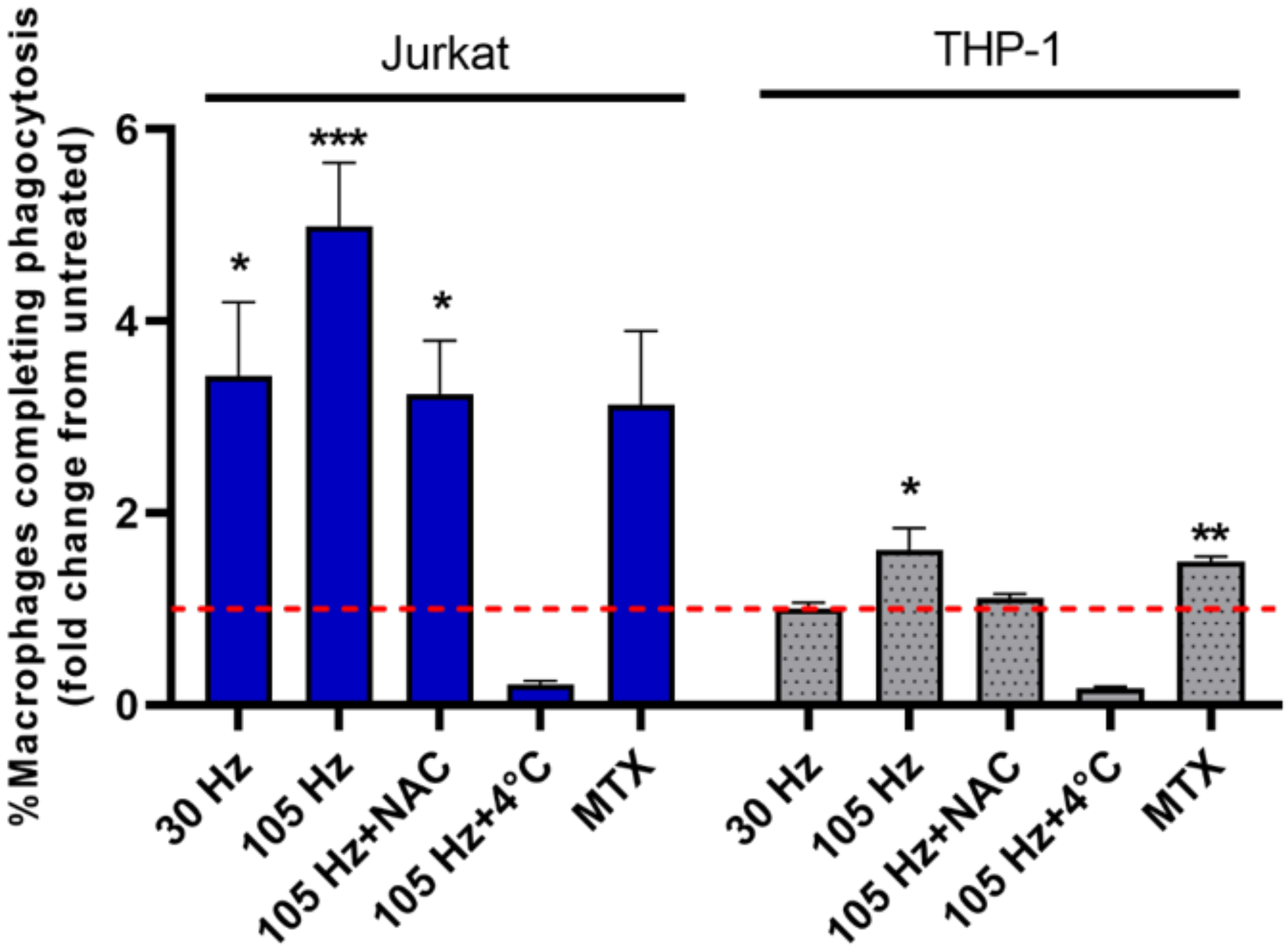
Phagocytosis



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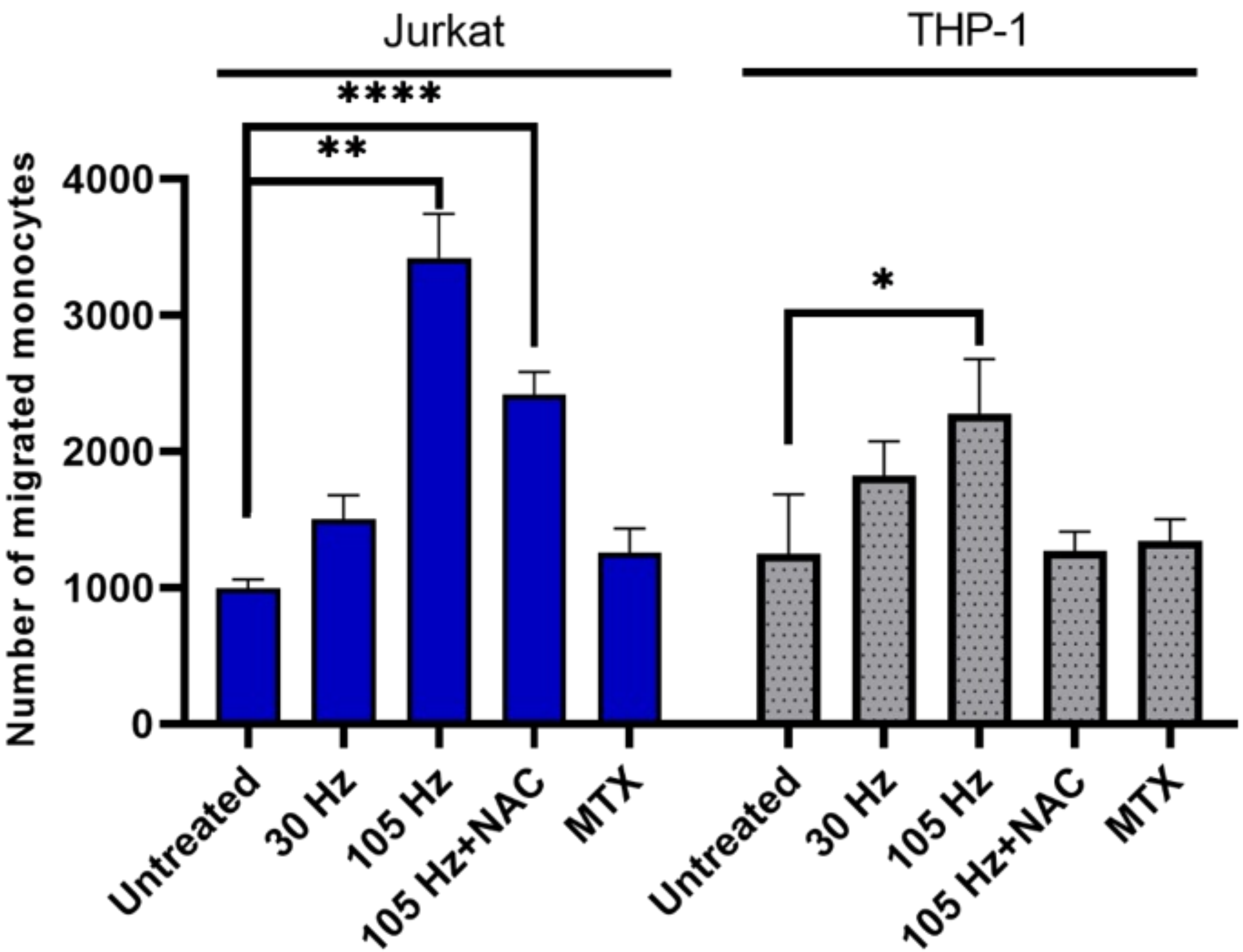
Stimulation of Monocyte Migration



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

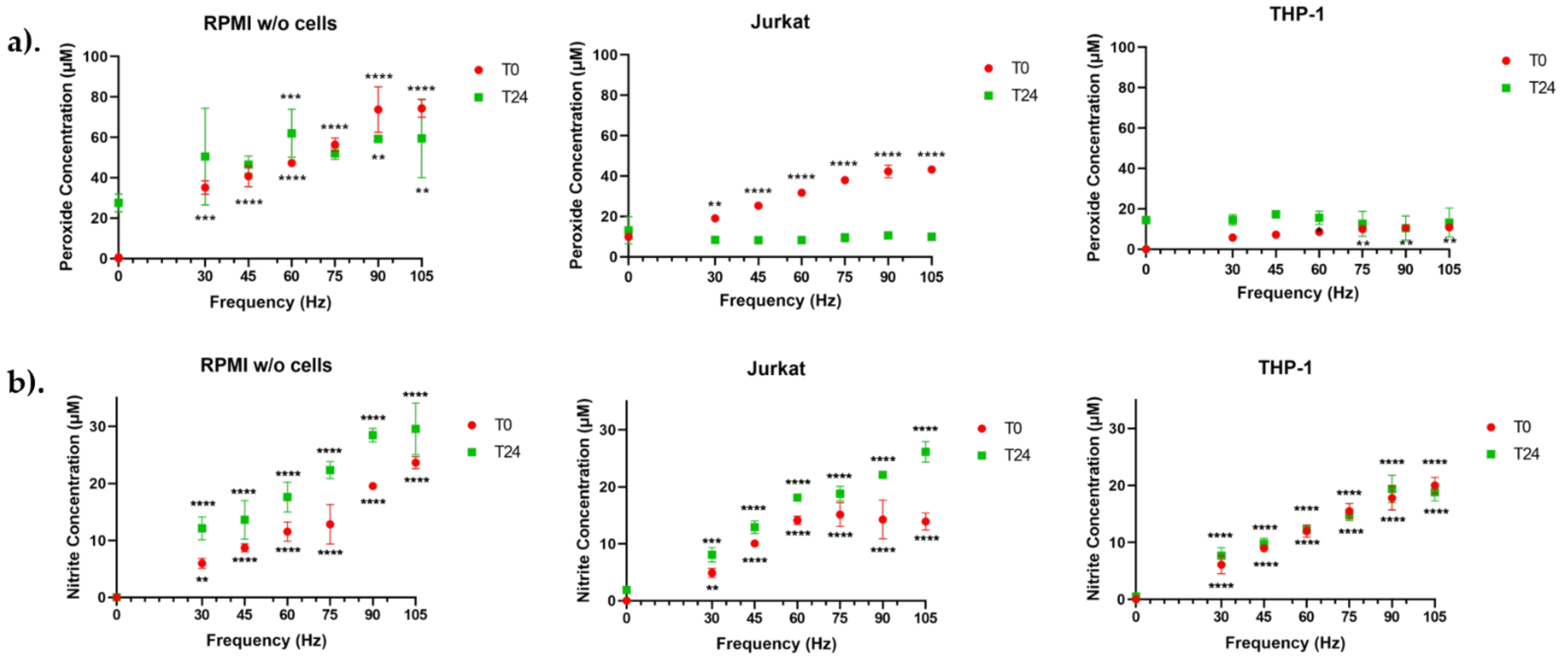
Summary of the Observations

- THP-1 cells are more resistant to NTP-mediated cytotoxicity
- Nonetheless, THP-1 cells showed increased level of chemotaxis and phagocytosis
- Phagocytosis without cytotoxicity may open up new avenues for plasma oncology

- Can the plasma chemistry help shed light on the two different cell responses?

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

Plasma-Liquid Chemistry with Cells



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

Plasma-Liquid Chemistry in the Presence of Organic Matter

- Cells influence the plasma-liquid chemistry in two ways:
  - Passive: they just provide a target to react with
  - Active: cells contribute to the chemistry (superoxide production) or take up reactive species to neutralize them (THP-1 cells)
- Closer look at reaction targets in liquids

COST Reference Microplasma Jet

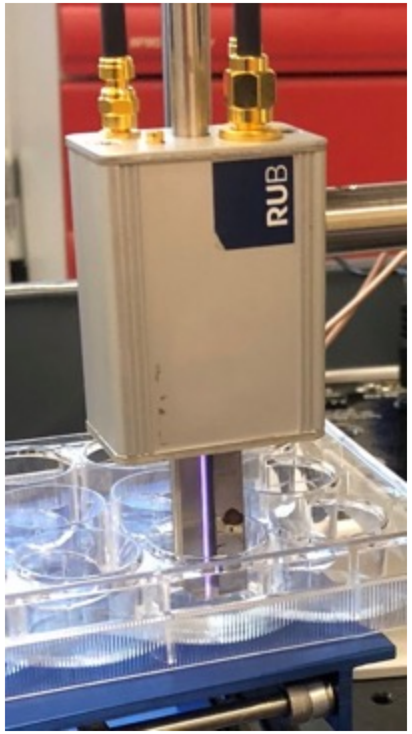
COST Reference Microplasma Jet

- 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

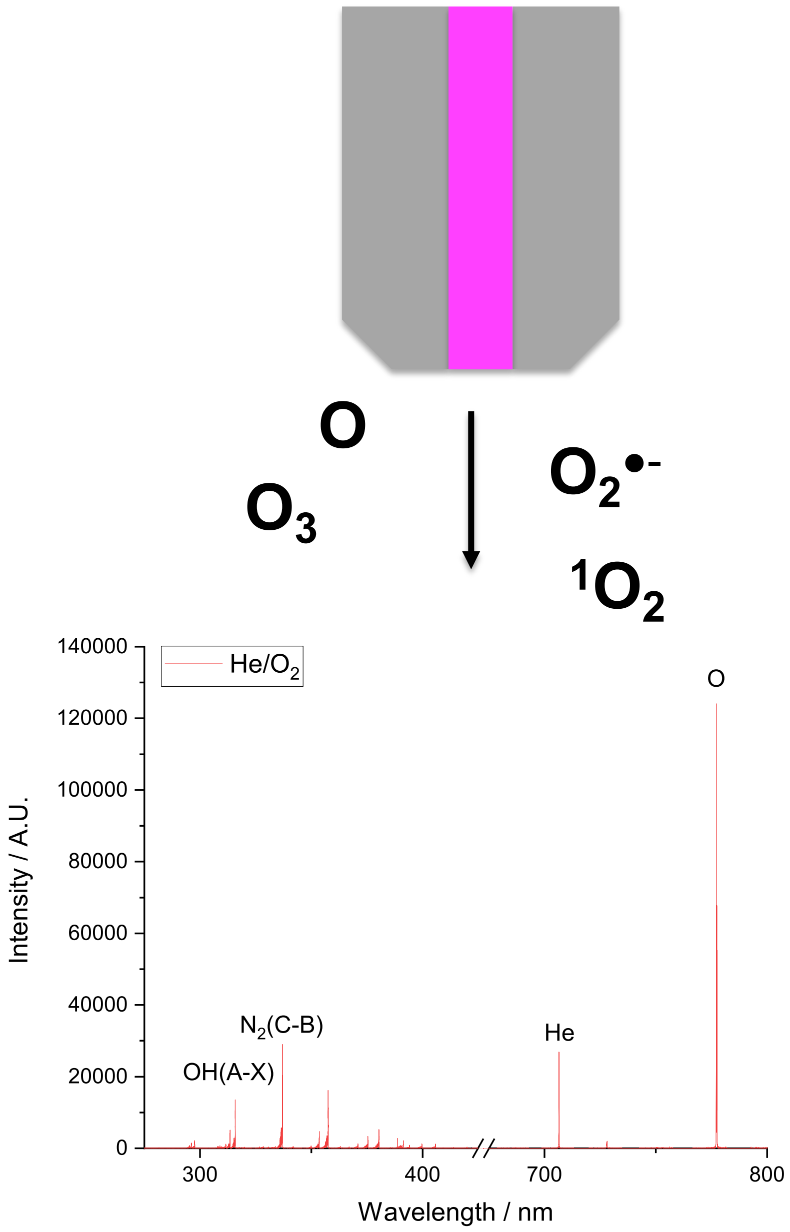
Golda J et al. Journal of Physics D: Applied Physics. 2016;49(8):84003.

Experimental Conditions

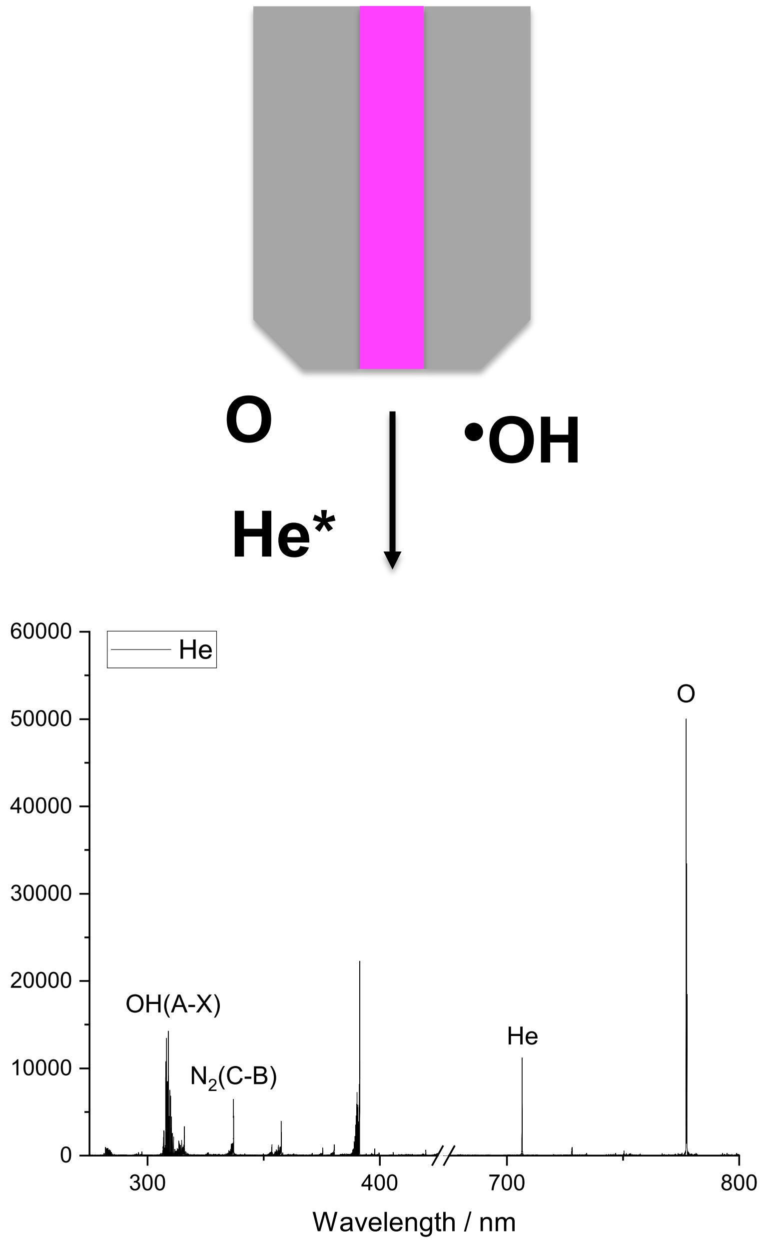
- Total gas flow: 1 slm
- Power constant at 750 mW
  - ~240 V_{RMS} for He/O_2 and He/H_2O
  - ~215 V_{RMS} for He
- Liquid treatments:
  - 12-well plate, 1 ml treatment volume
  - 4 mm distance from nozzle to liquid
- All measurements performed in triplicate



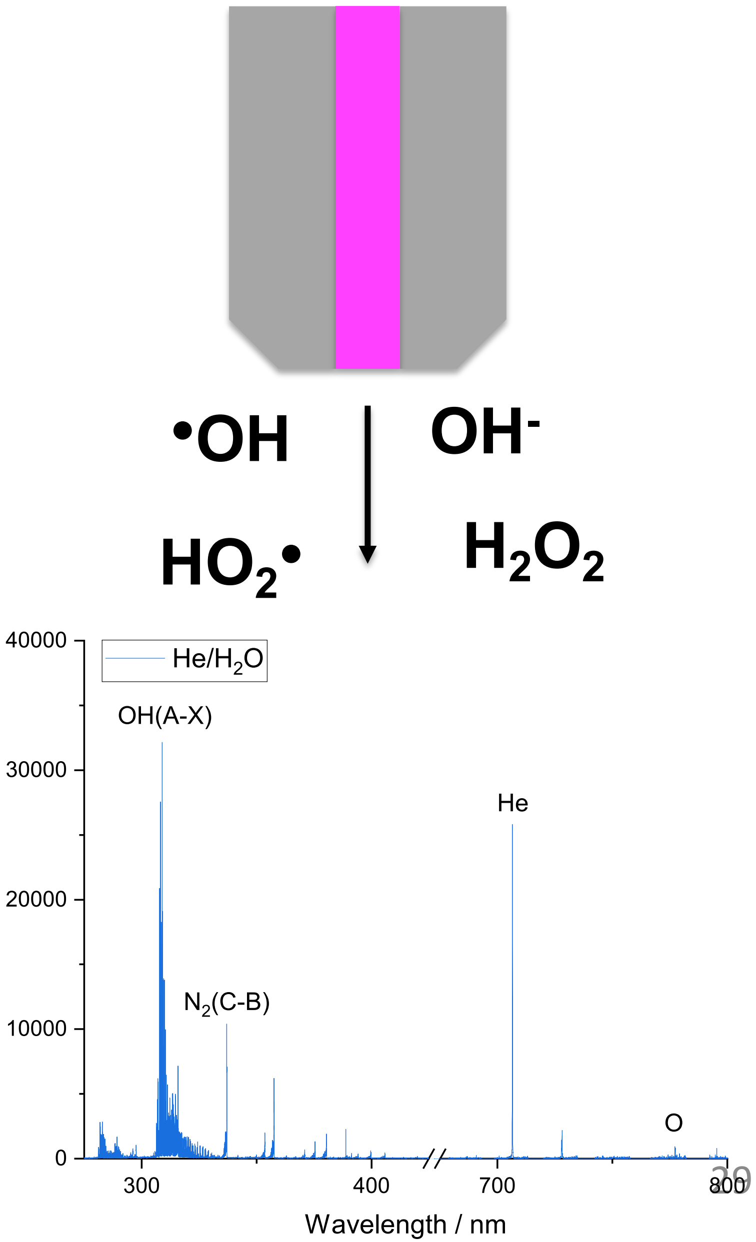
He 0.6% O_2



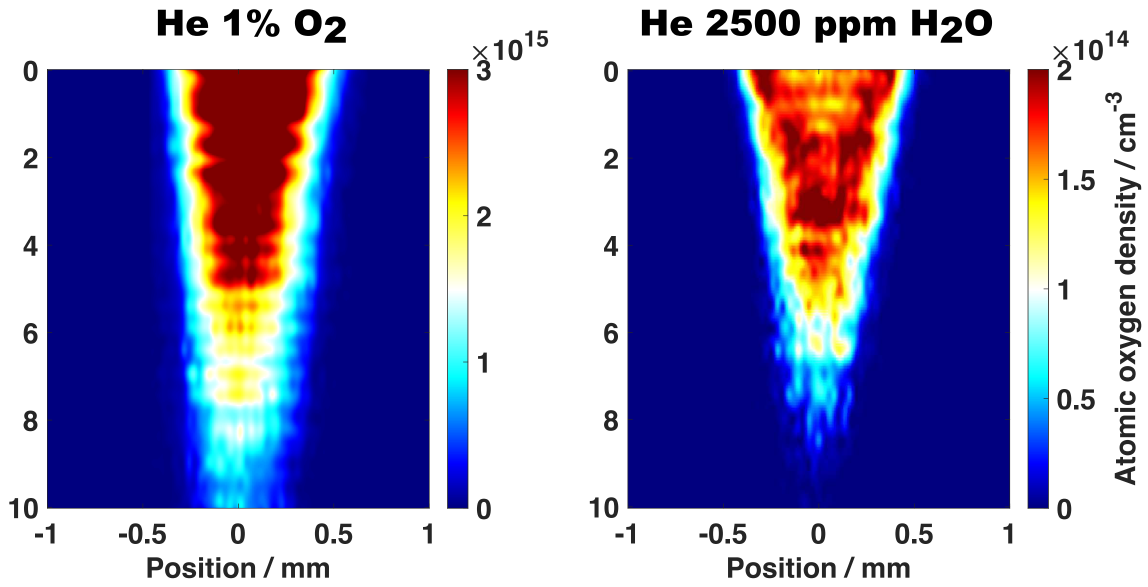
He-only

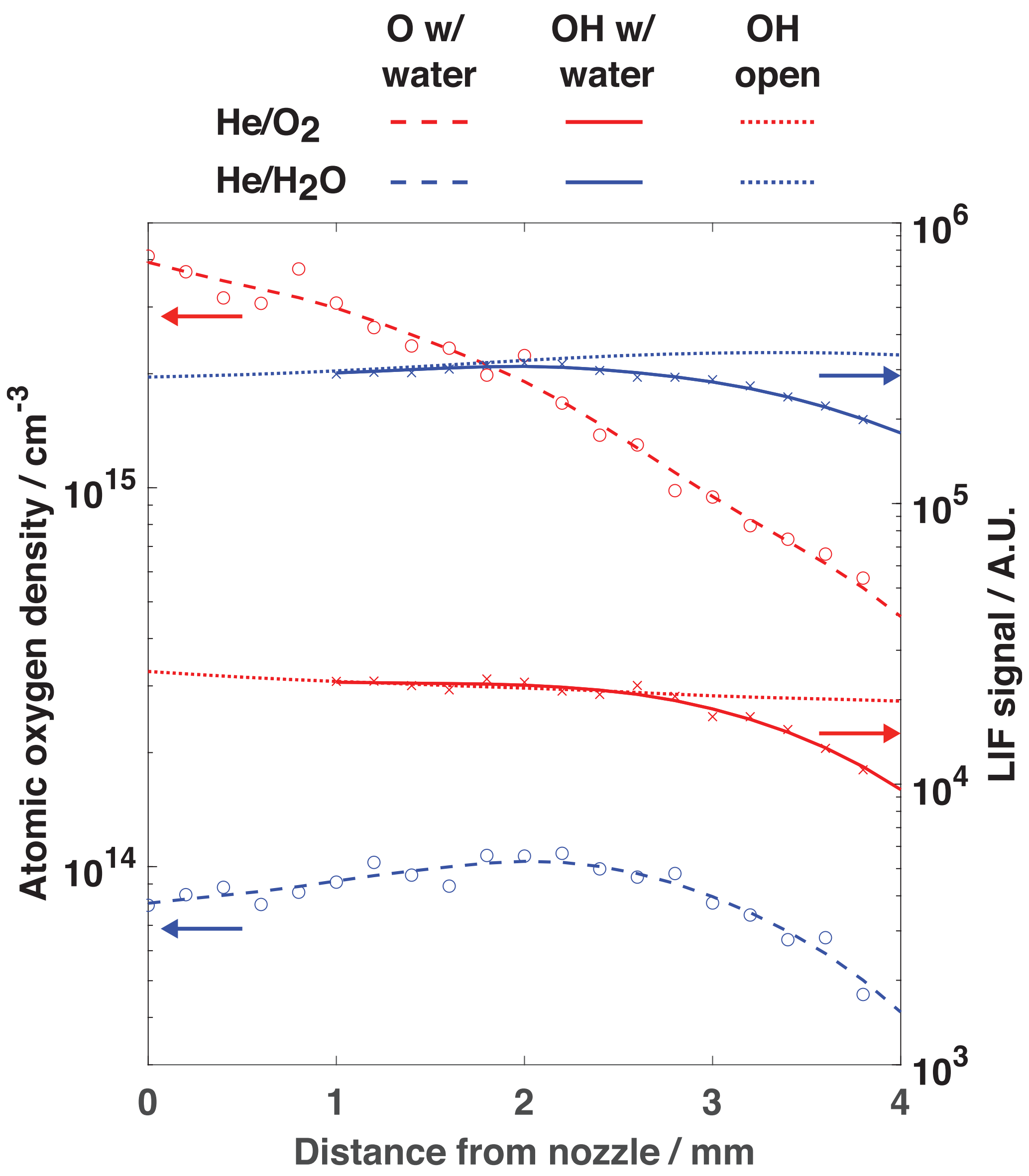


He 2500 ppm H_2O



O and OH in He/O_2 and He/H_2O

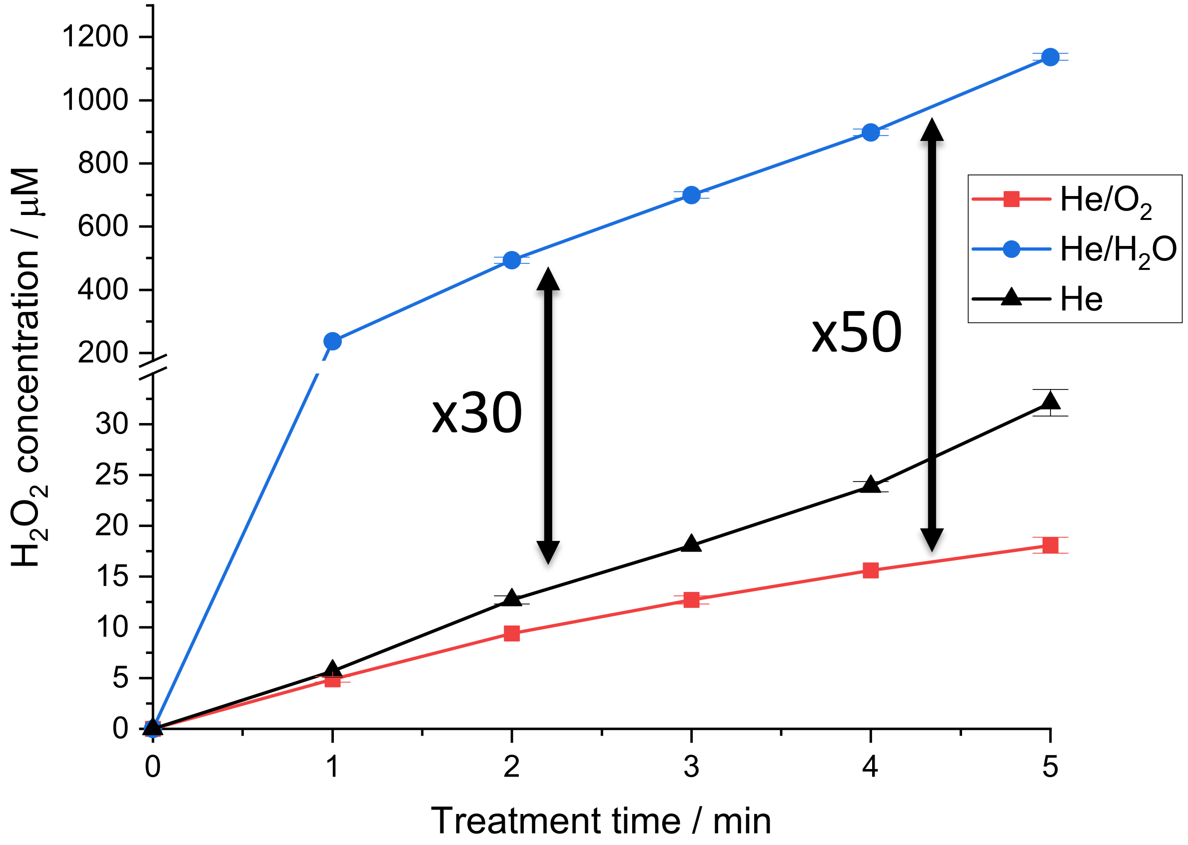




- O (TALIF) and OH (LIF) in an open effluent and with a liquid interface present at 4 mm distance
- O dominates in He/O_2, OH in He/H_2O

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



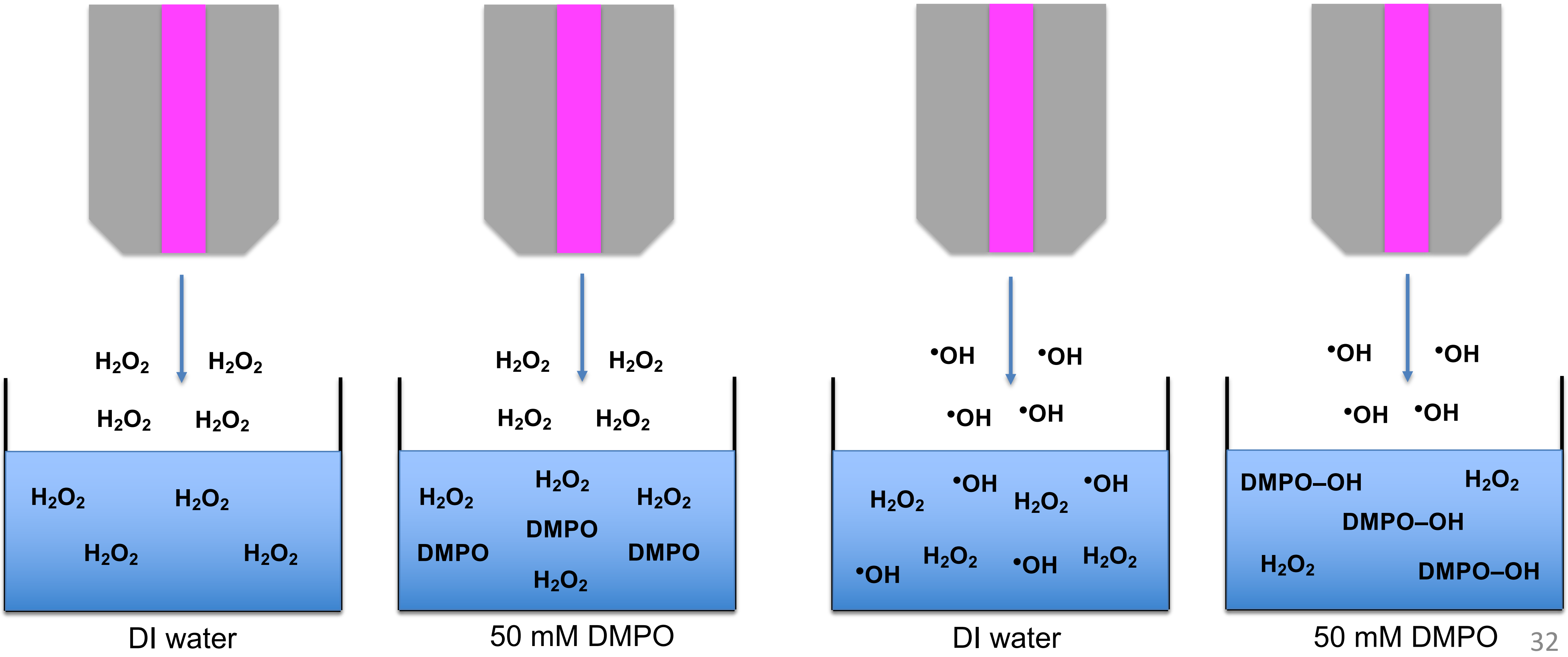
- H_2O_2 in He/H_2O plasma-treated sample is ~30x higher than He, ~50x higher than He/O_2
- Corresponds well to OES and previously reported gas phase measurements*

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

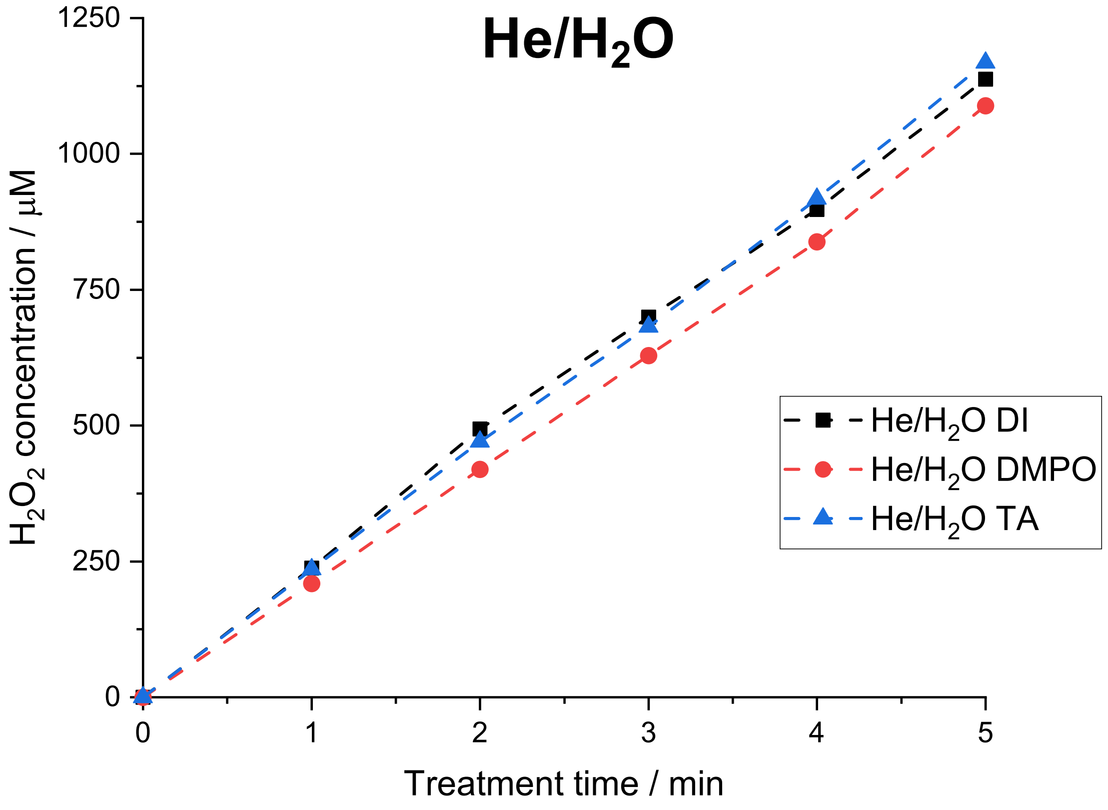
Isolating H_2O_2 origins using OH scavenger

H_2O_2 is produced primarily in gas phase

H_2O_2 is produced primarily in aqueous phase



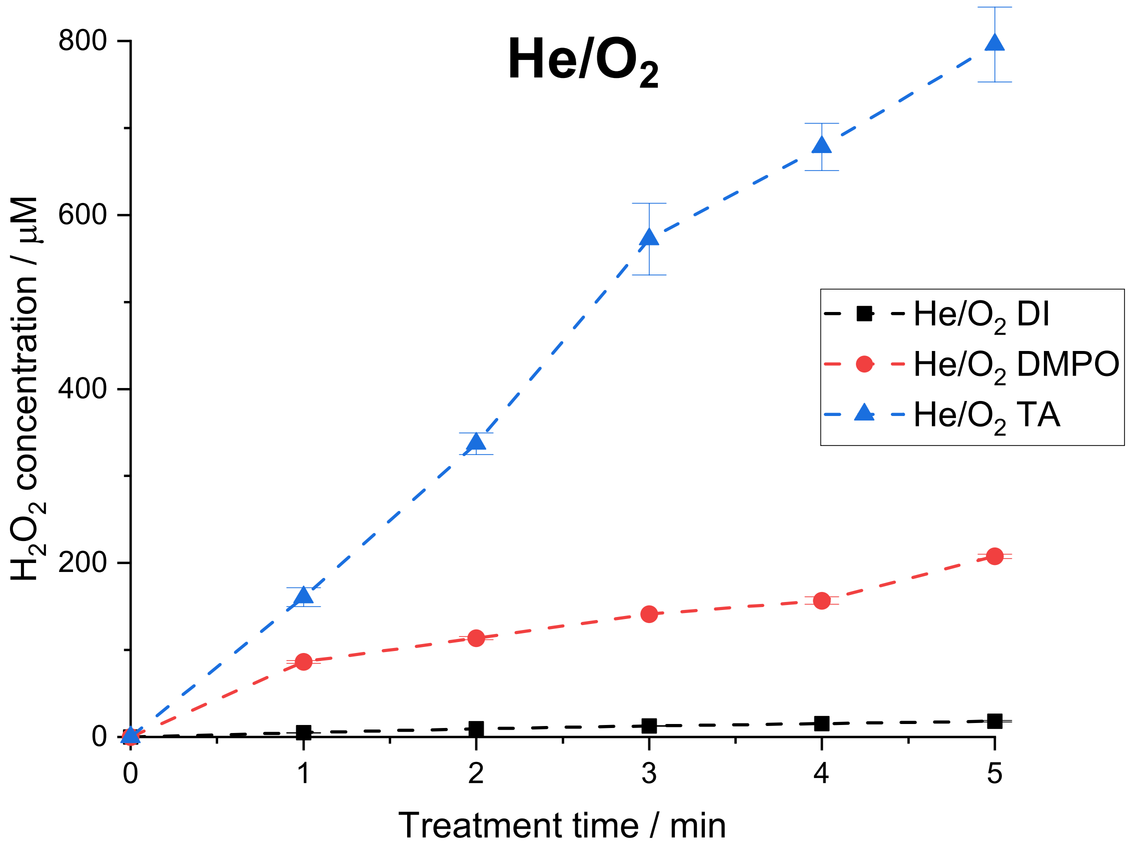
Isolating H_2O_2 origins using OH scavenger



- 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*

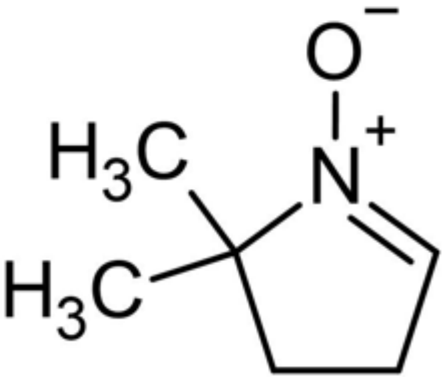
Isolating H_2O_2 origins using OH scavenger



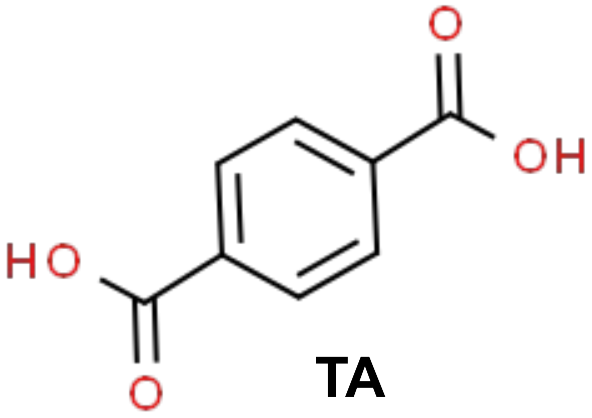
- H_2O_2 varies significantly between solutions
- Observed previously in phenol*
  - H abstraction by O from C-H bonds
- O enters liquid and reacts further to form H_2O_2
  - OH or HO_2 as precursor?

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.

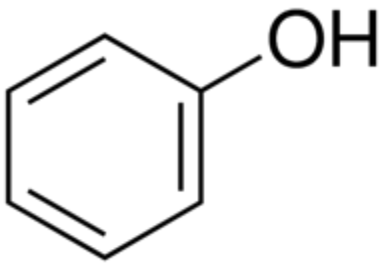
Comparison DMPO, TA, Phenol



DMPO



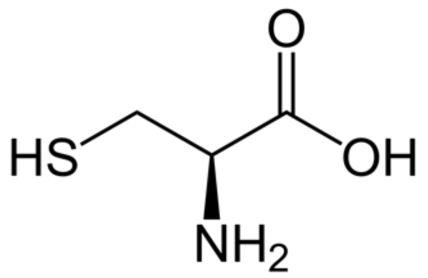
TA



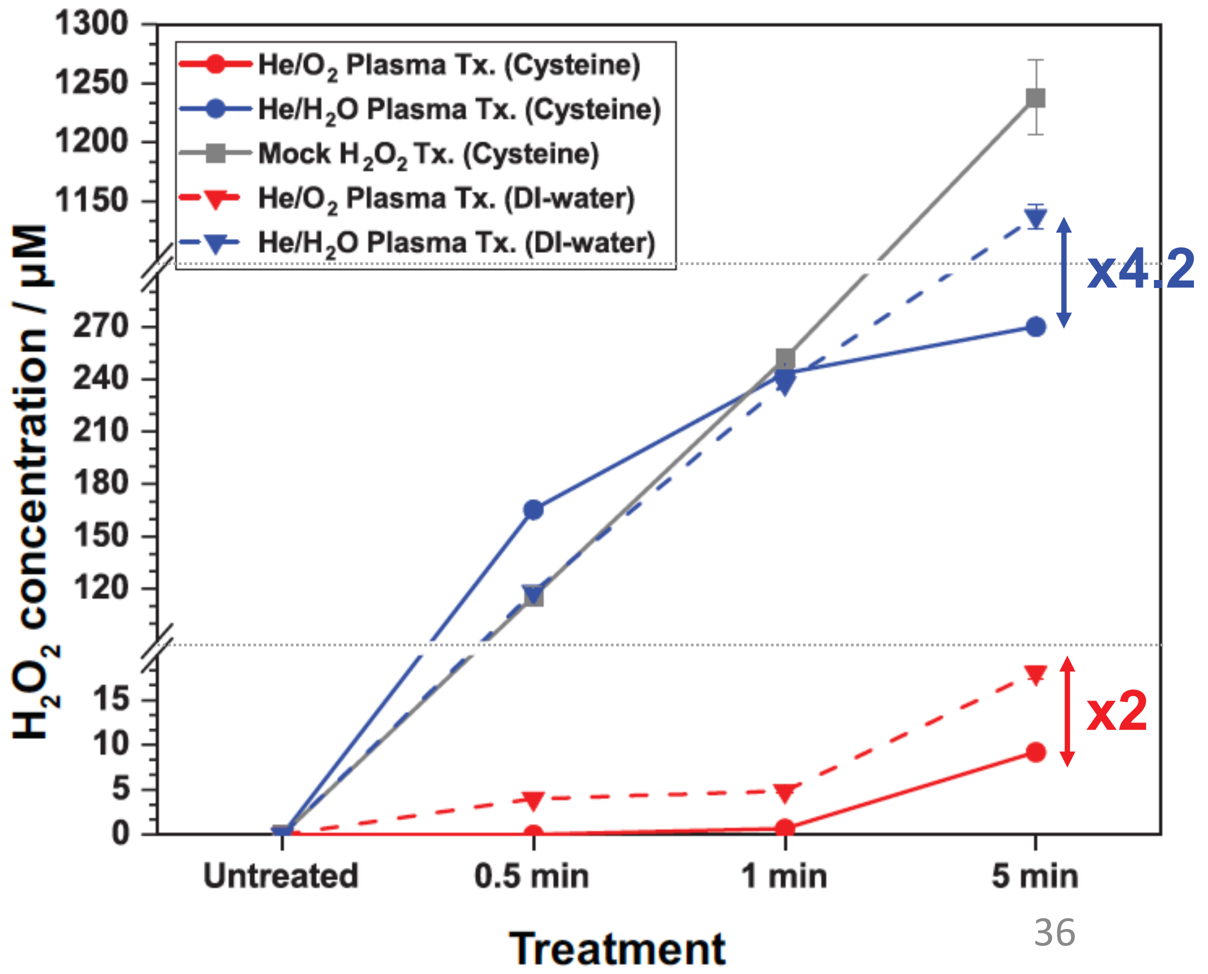
Phenol

- 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

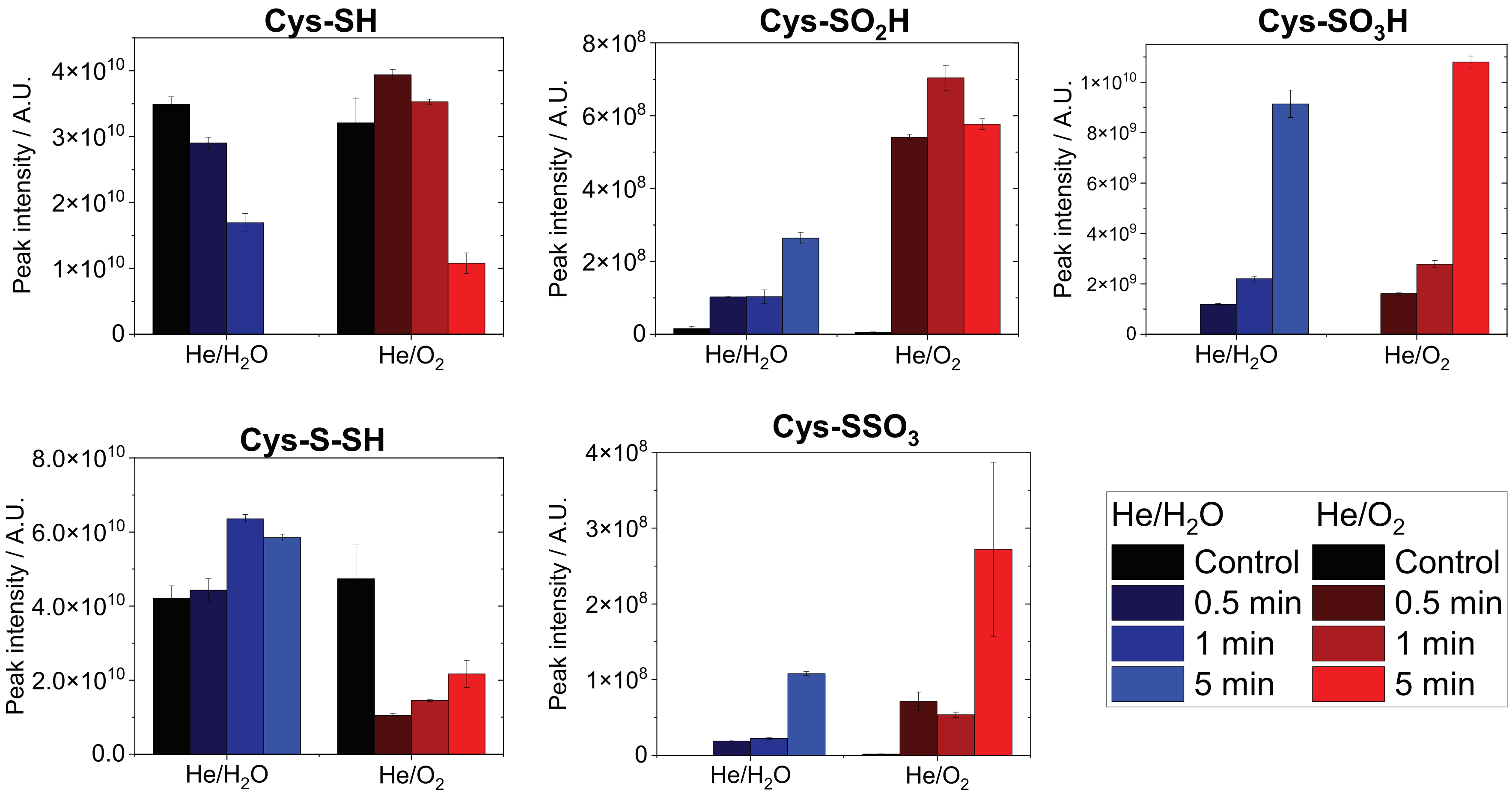


- Amino acid cysteine as simple model
- He/H_2O
  - up to 1 min.: DI ~ cys ~ cys + H_2O_2
  - 5 min: mock > DI >> cys
  - Cys consumed H_2O_2
  - Not if only H_2O_2 is present: short-lived species necessary to initiate reactions
- He/O_2
  - DI water higher H_2O_2 concentration
  - Cys consumed H_2O_2



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

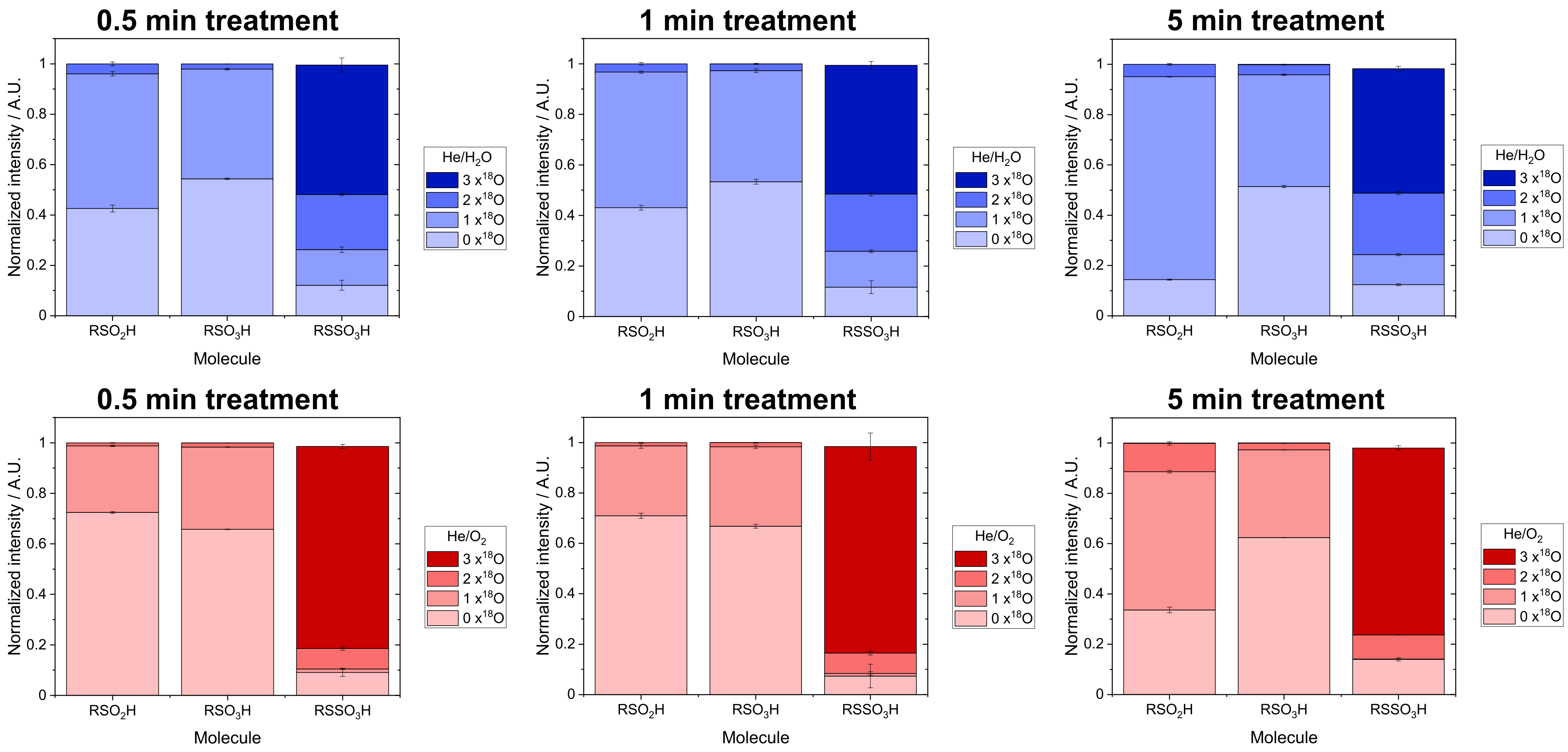
Cysteine Modifications



- 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

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

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)

Challenges and Opportunities

- Modifications on biomolecules can be tuned by using different plasma sources, voltage conditions, gas admixtures
- Modifications *known to nature* vs *unknown to nature*
  - OH/H_2O_2-driven chemistry vs O-driven chemistry
  - Reversible vs irreversible modifications in nature
  - Translation from single amino acids to organisms?
- RONS produced by plasma and in the context of redox biology
  - Precise manipulation of cellular responses possible?

Outlook – ongoing research

- Identification of short-lived species in the liquid by EPR spectroscopy
  - NO, OH, O in water and cell culture medium
- Impact of NO-, OH-, and O-rich plasma on cell culture
- Response of cells to plasma treatment – different cellular markers and cell-produced chemistry
- Can we use plasma and cell-produced chemistry for ”plasma endpoint detection”? → new NIH project

UNC LINEBERGER COMPREHENSIVE CANCER CENTER

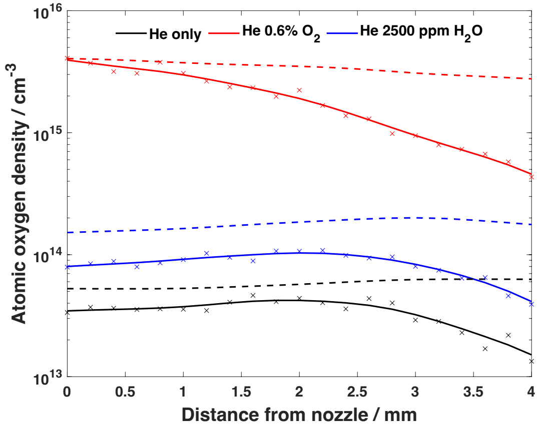
NIH National Institute of Biomedical Imaging and Bioengineering

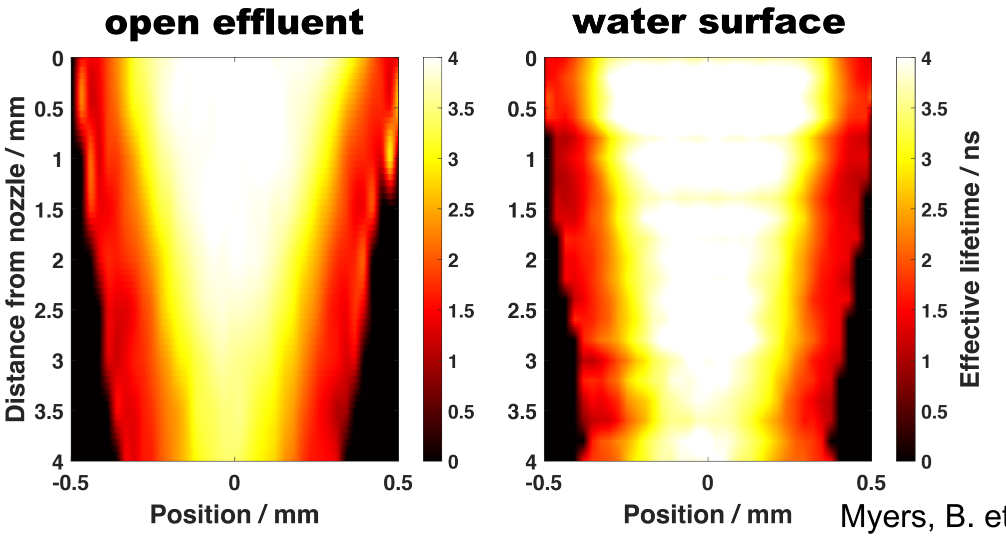
Research Areas
Plasma for Life Sciences

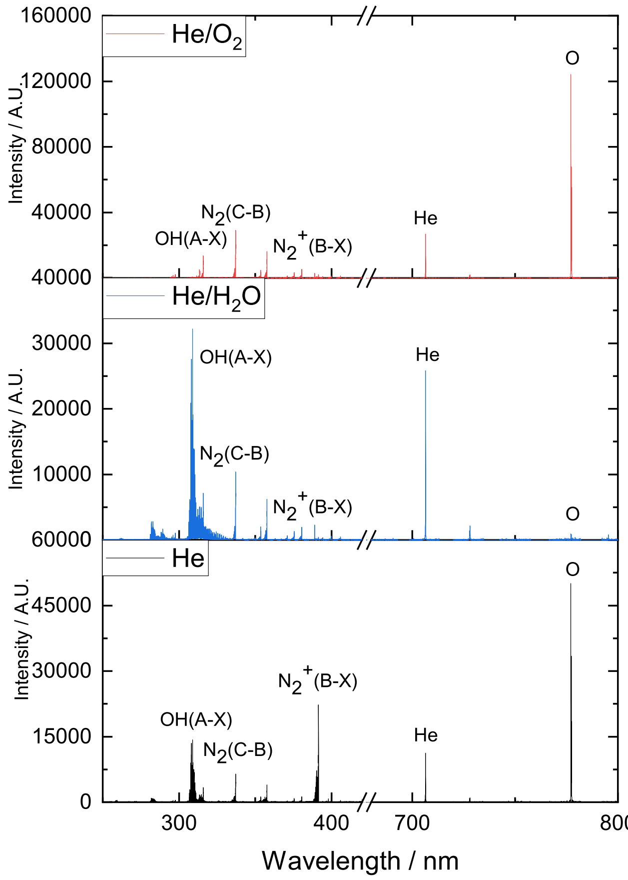
Generation & Transport of Reactive Species - from the gas phase to the liquid to biological samples

COST jet:

Plasma chemistry: what is produced and where, how does it interact with liquids, biological samples, …







U.S. DEPARTMENT OF ENERGY

DE-SC0021329

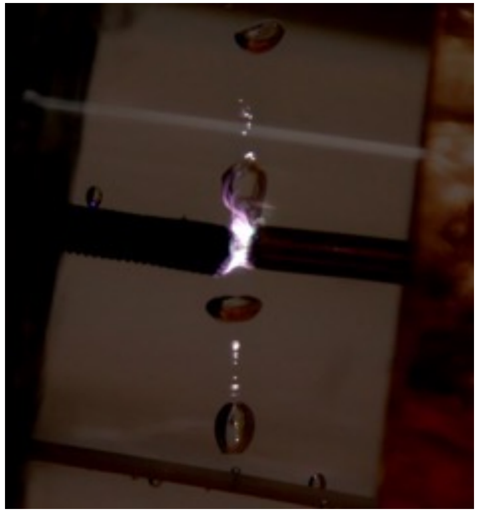
Myers, B. et al. *Journal of Physics D: Applied Physics* **54** (2021): 145202

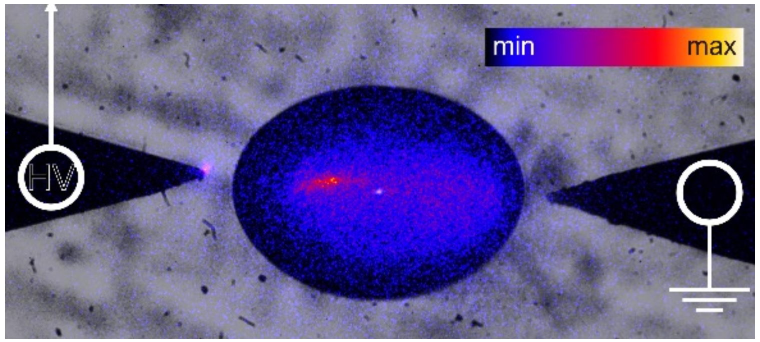
Plasma Breakdown and instabilities in the multiphase plasma-gas bubble-liquid system

Plasma Bubble Reactor for Water Treatment:

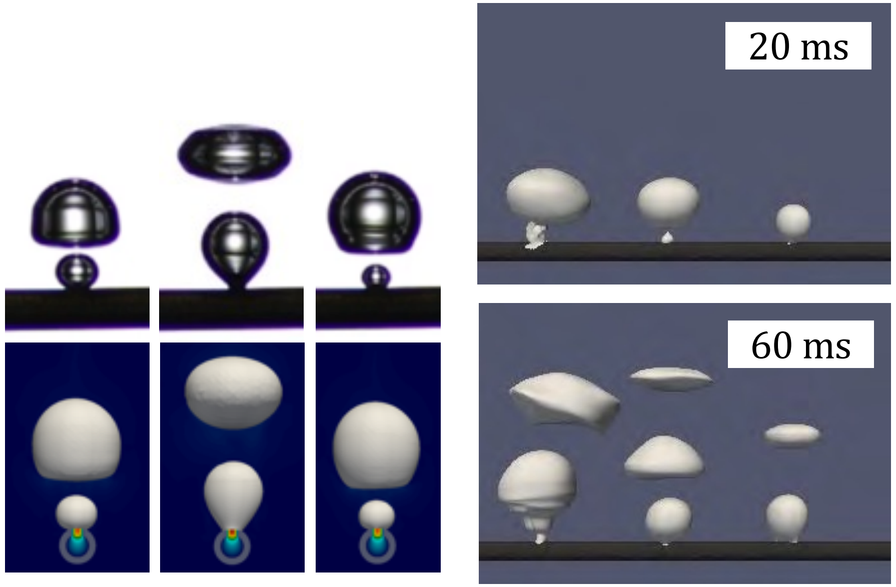
Understanding breakdown and streamer development in plasma bubbles – experimental & theoretical approach

PHY 2107901

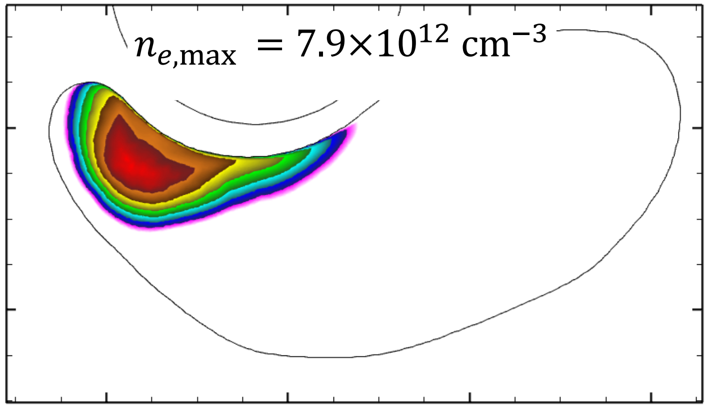




Experimental investigation

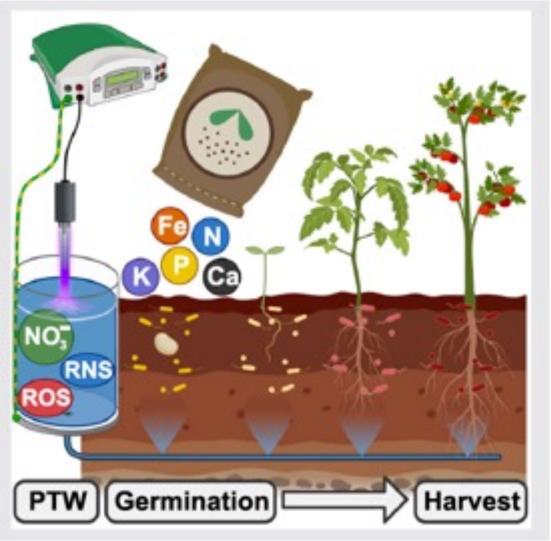


DNS bubble formation

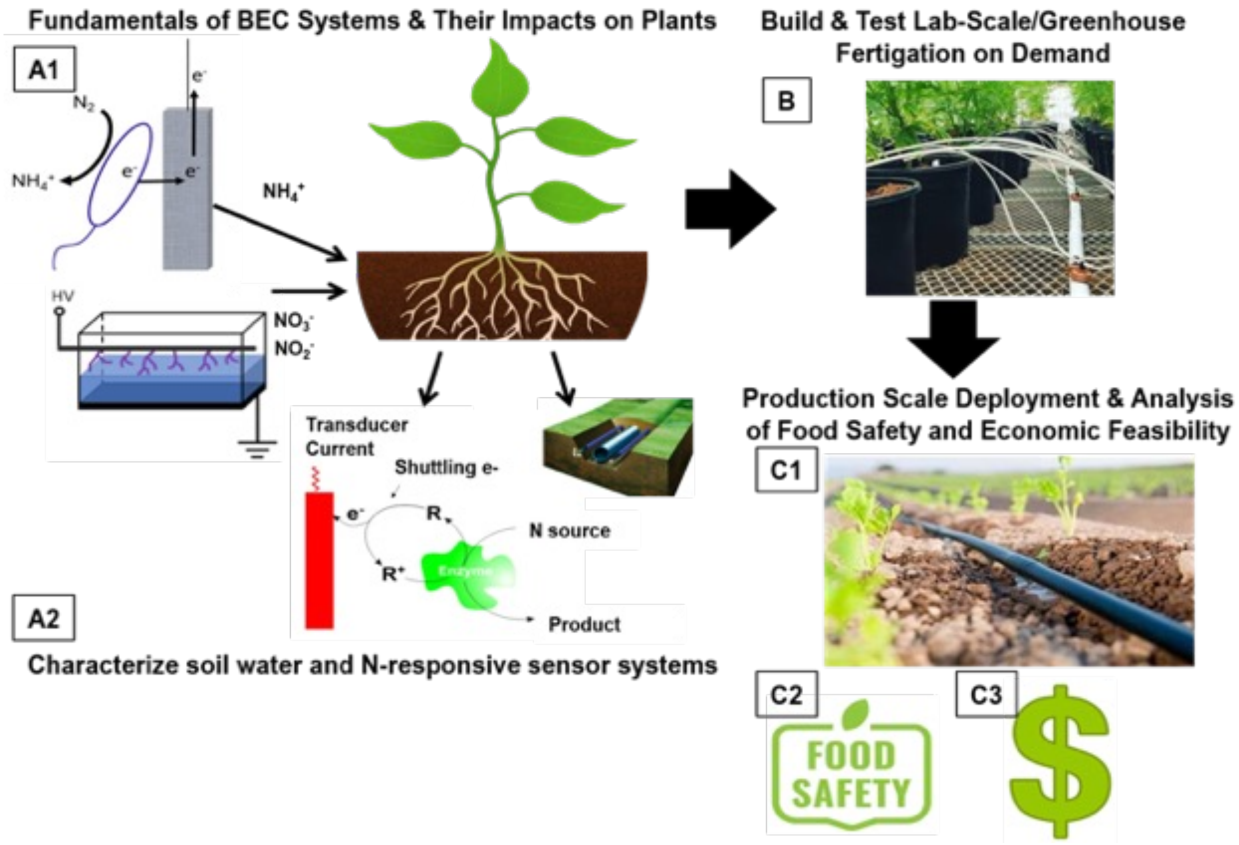


Bubble shape - nonPDPSim

Plasma Agriculture



"Fertigation on Demand" - Plant Sciences Initiative @ NCSU



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REVIEW

PLASMA PROCESSES AND POLYMERS

Plasma agriculture: Review from the perspective of the plant and its ecosystem

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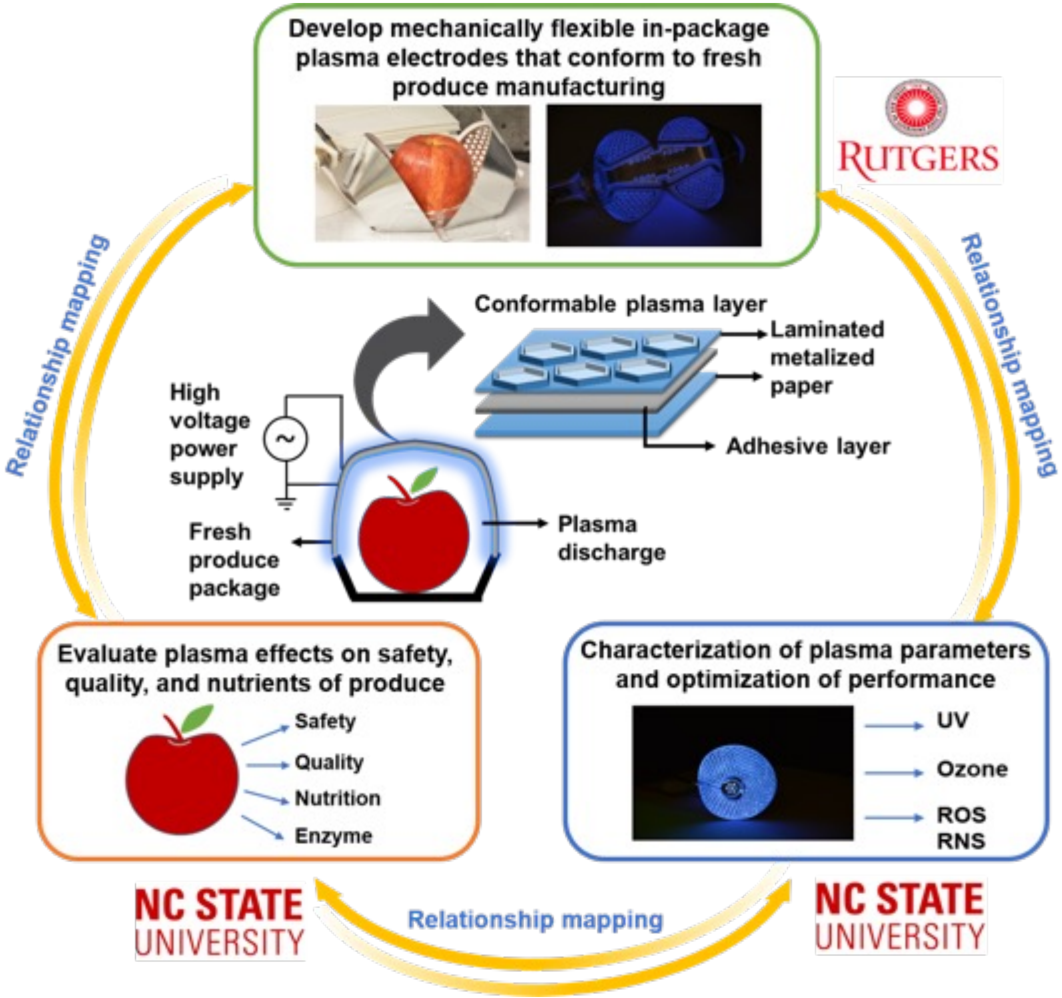
Plasma Agriculture II

Flexible DBD for treatment of fresh produce:



High-quality manufacturing of packaged fresh produce with conformable in-package cold atmospheric plasma,

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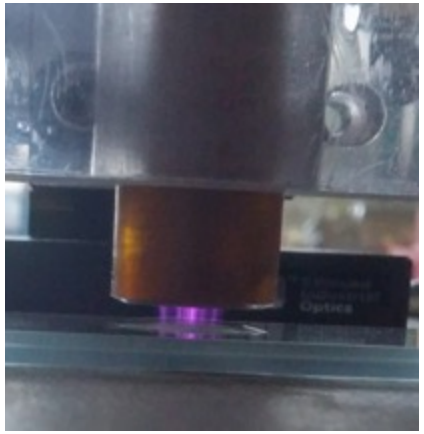

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