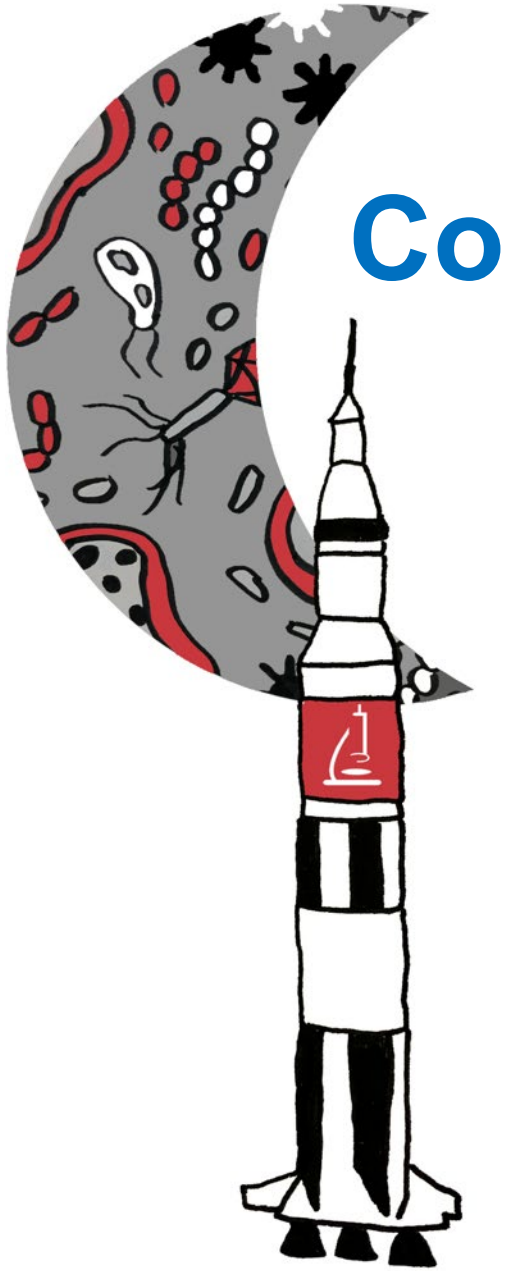

Cold plasma as cleaning technique for planetary protection

Tanya Sysoeva

@MicroRocket

UP Lab

BIOLOGICAL SCIENCES

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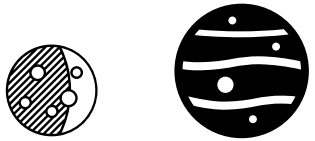
Sarah Adkins

Standard sterilization processes

- high temperature (bake out, autoclaving) & radiation (ionizing, UV)
- specific or broad chemicals – antibiotic vs disinfectants (harsh chemicals)
- liquid (bleach, alcohols) vs gaseous (peroxide, ethyl oxide)

Planetary protection

- Goal: to prevent the contamination of celestial bodies by Earth life and vice versa
- Long term human habitats on Moon and Mars
  – no resupply but returns



to the Moon (to Mars) and back

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Some Planetary Protection Help
1 message

*Planetary Protection = not contaminating other planets/moons/asteroids with earth-born organisms and viceversa.

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Research project
4 messages

investigating use of low-temperature gas plasmas for disinfection and sterilization.

John Mayo

Cold plasma against microbes

- What Is Plasma? - Fourth state of matter
- 99% universal baryonic matter
- **Ionized gas** with a significant amount of free electrons and charged ions
- Thermal and **non-thermal "cold" plasma**

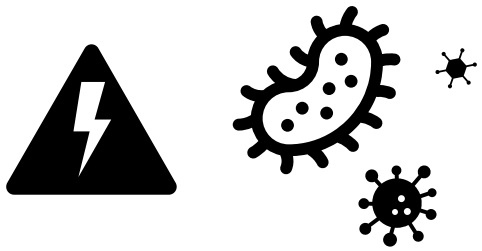
"Northern lights" in North Alabama

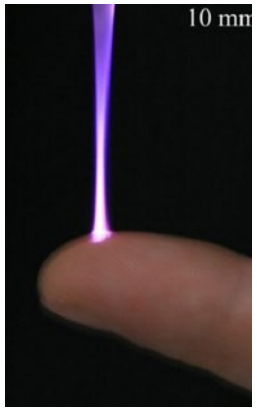
Gabe Xu

Mechanical and Aerospace Engineering, UAH

Cold plasma against microbes

- **Microbicidal** for whole host of tested bacteria, fungi, viruses
- Cold plasma forms reactive oxygen and reactive nitrogen species (RONS) (*and UV*)





DOI: 10.1088/0022-3727/44/10/105204

Considerations for Use of Low-Temperature Gas Plasmas for Mitigation of Biofilms in Microgravity Environments. Bhatt K., Mayo J., Xu G., Ramsey D., Sysoeva T.A. 2021 for the National Academies Decadal Survey on Biological and Physical Sciences Research in Space 2023-2032

Can cold plasma be a specialty PP cleaning?

- Gentle for diverse surface materials
- Minimal chemical supplies (not a traditional liquid/gas sterilant) and created *in situ*

Lanie Briggs

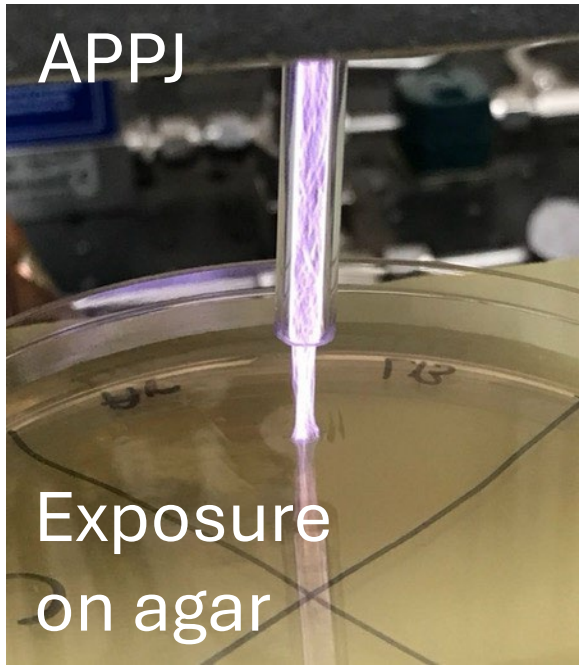
- **optimization of cold plasma for relevant organisms and materials – 2D**
- **testing cold plasma 'reach' for hardware with complex geometry – 3D**

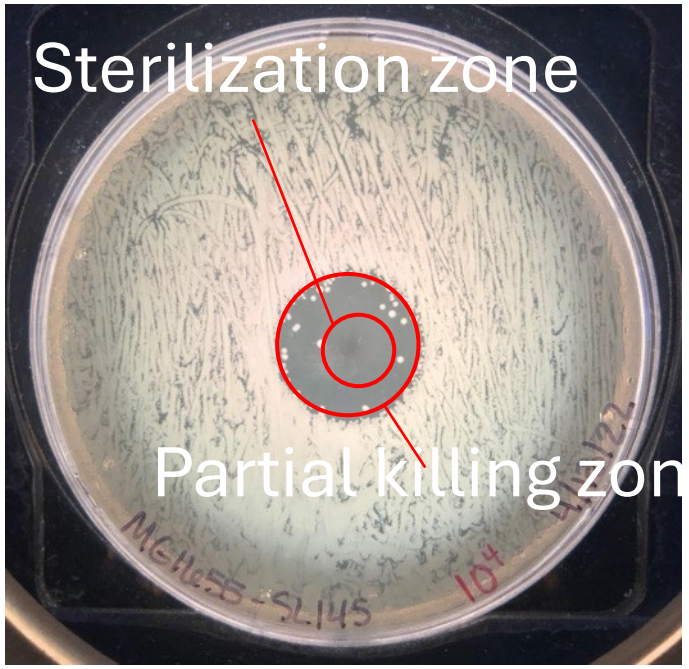
Bhagirath Ghimire

Considerations for Use of Low-Temperature Gas Plasmas for Mitigation of Biofilms in Microgravity Environments. Bhatt K., Mayo J., Xu G., Ramsey D., Sysoeva T.A. 2021 for the National Academies Decadal Survey on Biological and Physical Sciences Research in Space 2023-2032

Quantification of bactericidal efficiency

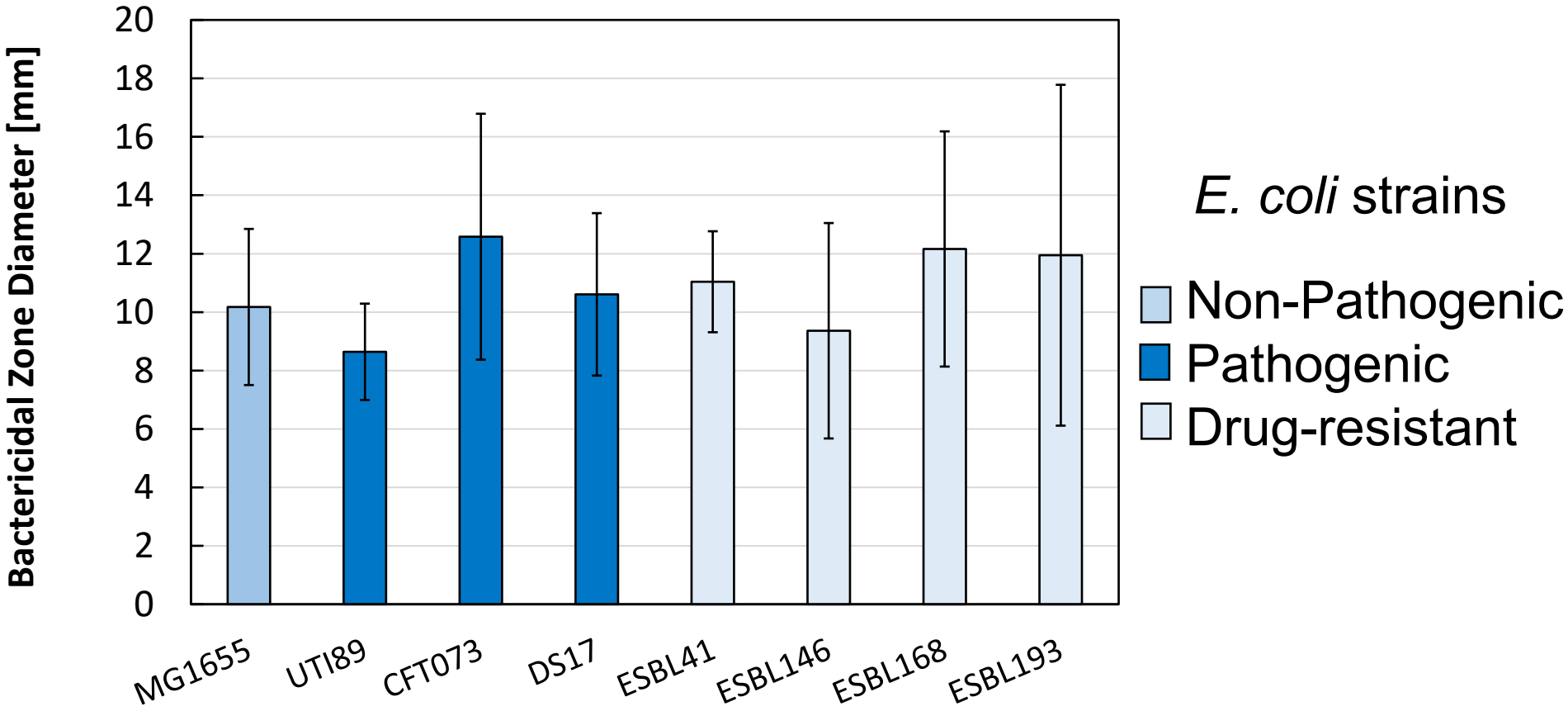
- bacterial cells on on agar support
- controlled parameters (cell density, type)
- operating parameters of plasma





2021 Seed grant from CPU2AL EPSCoR

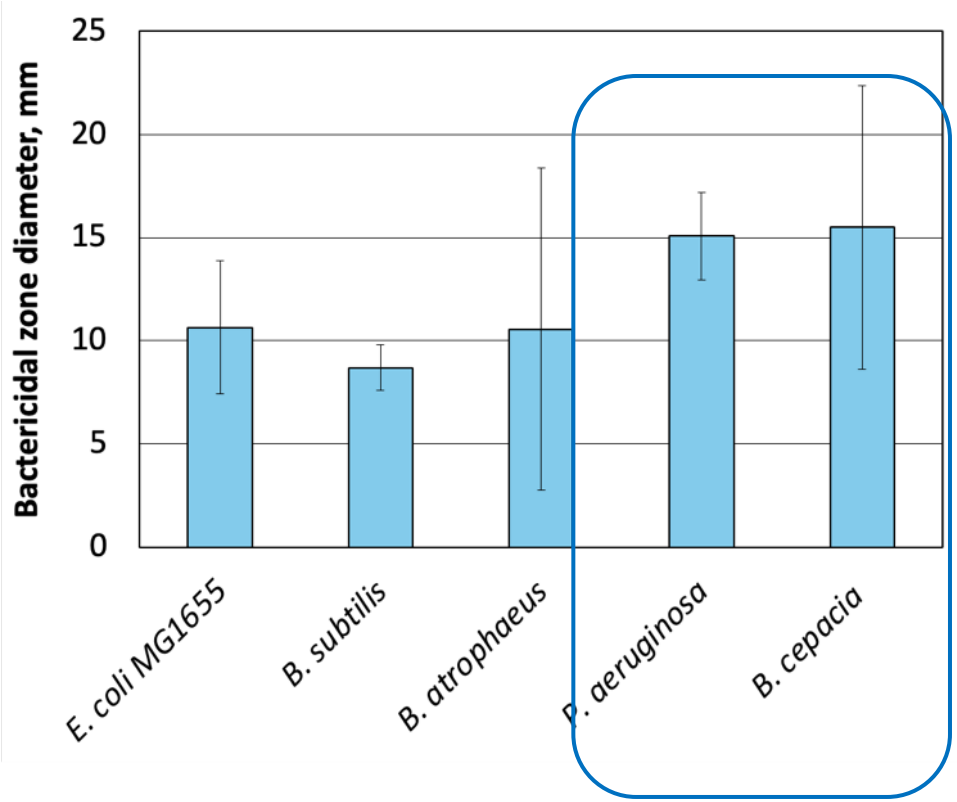
Multidrug resistant isolates are susceptible



2021 Seed grant from CPU2AL EPSCoR

Diverse bacterial species are susceptible!

- Gram positive and negative, spore forming organisms
- Hardy isolates from the ISS water recycling system



Yo Ann Velez Justiniano, MSFC

ISS isolates *P. aeruginosa* and *B. cepacia*

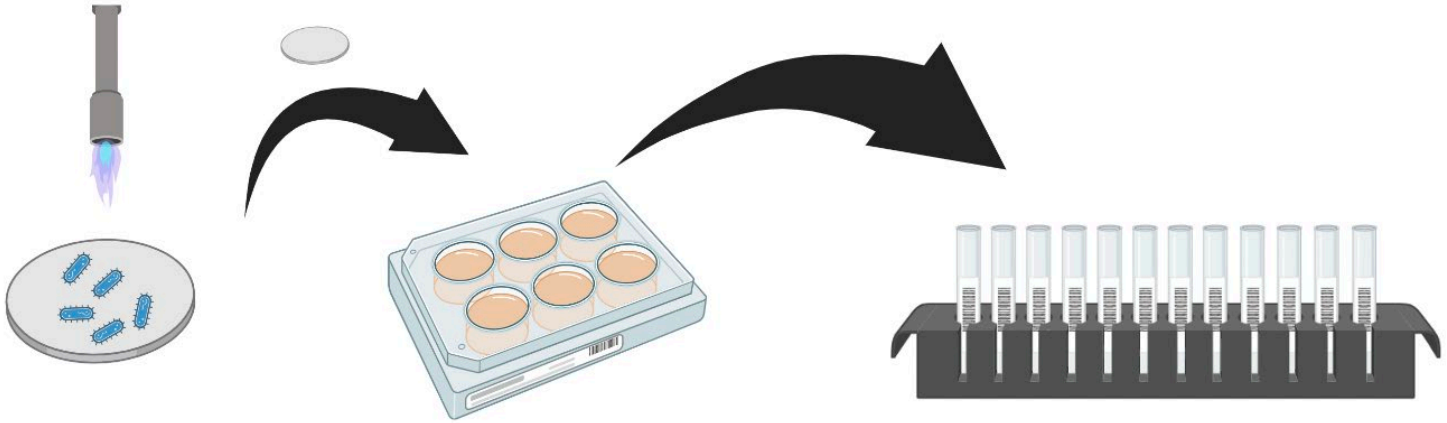
Velez Justiniano et al, More Than a Decade of International Space Station Microbial Sampling in the Environmental Control and Life Support Systems, 2024

Quantitative assay with solid coupons

- Different flight hardware-relevant materials
  metal & plastic
- Bioindicator targets
  B. atropheaus and *D. radiodurans*

Polycarbonate

Inconel 718

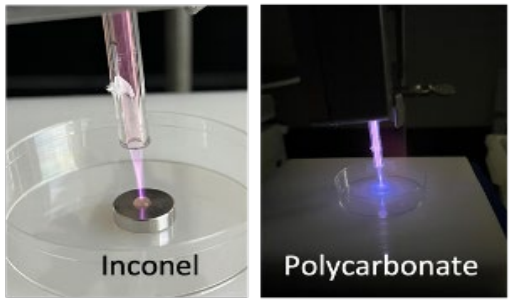


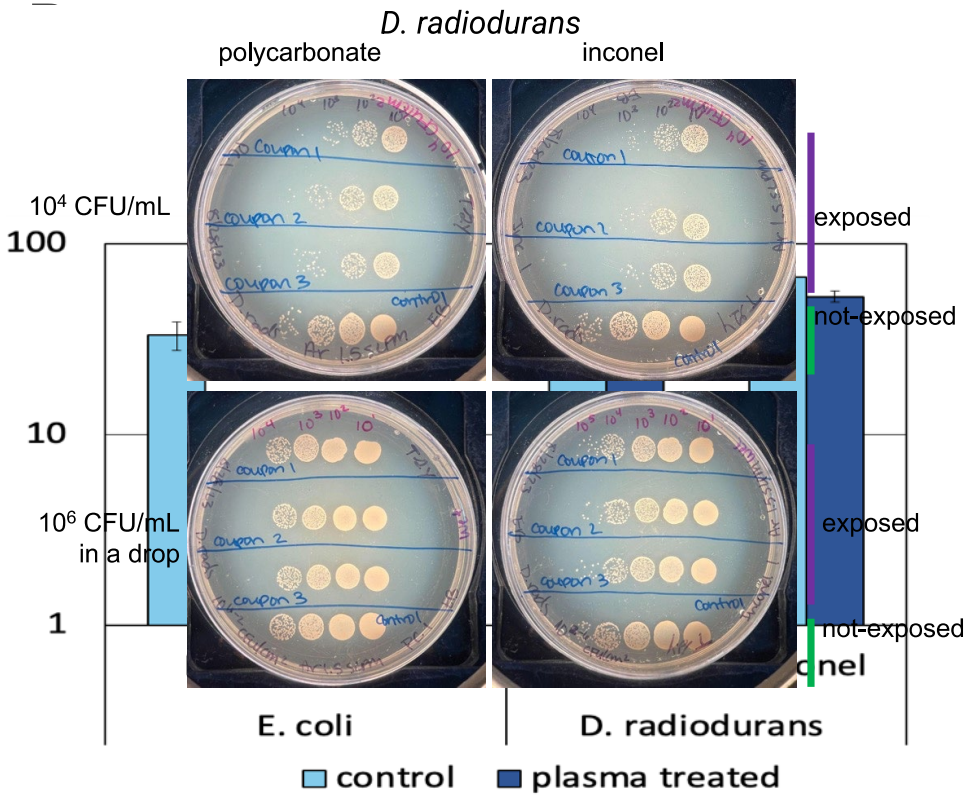
MSFC CAN award

Markedly reduced efficiency on dry coupons

- Both *D. radiodurans cells* and *B. atrophaeus* spores had only up to 1.5 order reduction in viability after plasma exposures analogous to on-agar tests

- Similar reduction was observed for less hardy organisms like *E. coli* and both types of materials





Markedly reduced efficiency on dry coupons

6 kV for 3 min

8 kV for 10 min

- Increase of time and voltage improved efficiency

- Wetness/humidity changes (drying time, salt/buffer) affect efficiency

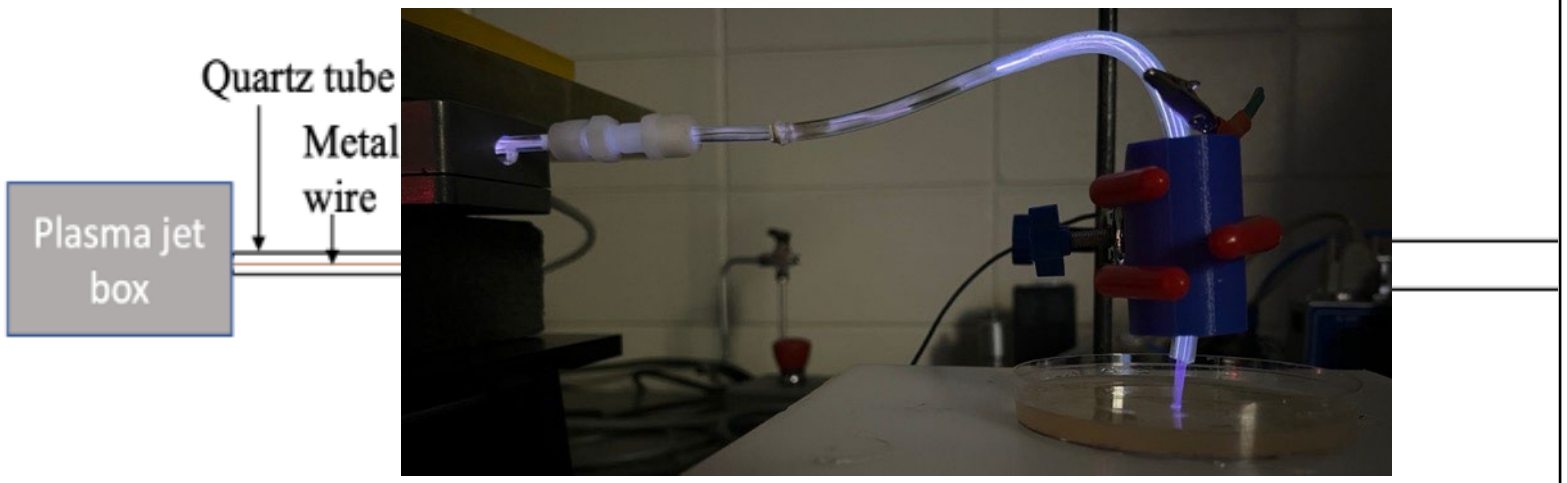
Mechanism for cold plasma on dry coupons

- Cell state and/or Lack of water?
- MS analysis to identify and quantify reactive species *in situ* using ACL capabilities

Ryan Gott, KSC

Narrow tubing propagation

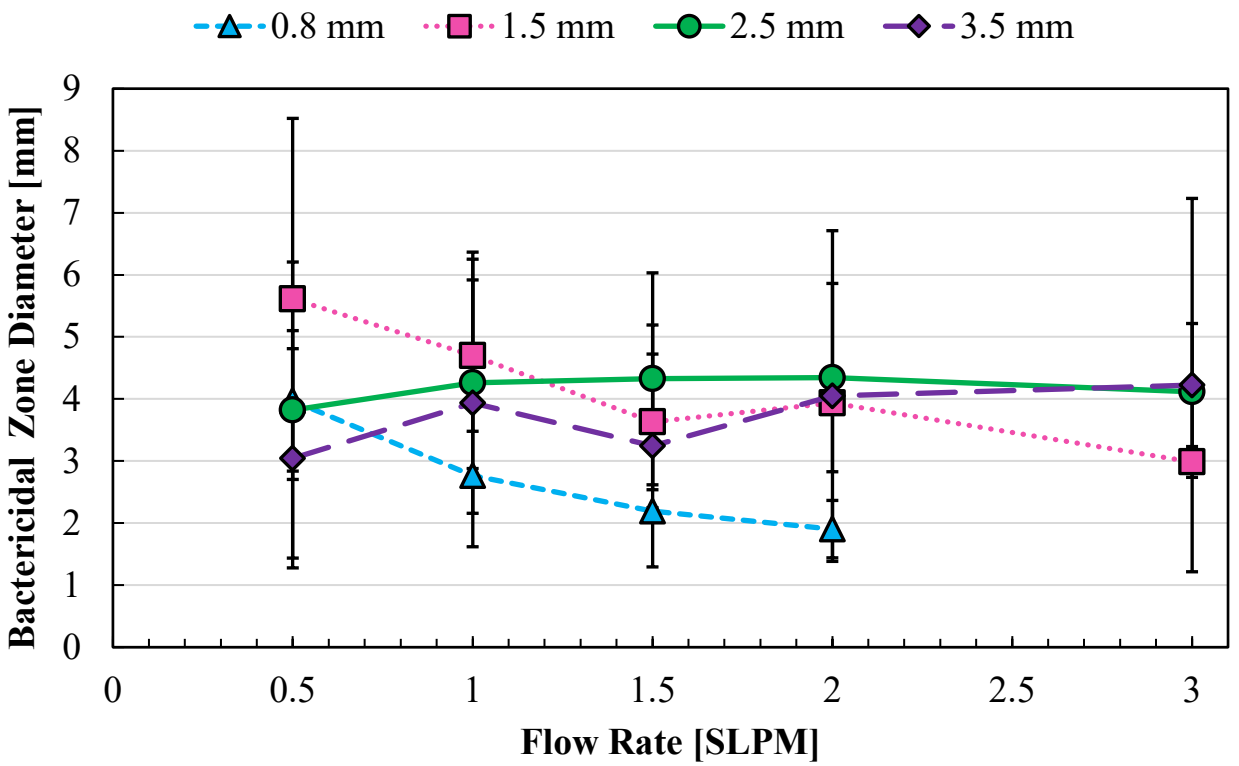
- 0.8-3.5 mm ID tubing (~200 mm length)
- Testing ‘outlet’ efficiency on exit of the tubing
- Model *E. coli* bacteria



Setup for CAPP propagation in tubing

Narrow tubing propagation

- Cold plasma successfully propagates through narrow tubing
- Bactericidal effect is retained at the tube outlet



Take home message

- Cold plasma works on wet surfaces such as agar on all tested organisms
- Bactericidal efficiency is strongly reduced on dry solid surfaces possibly due to lack of humidity or cell state
- Plasma can propagate through narrow tubing retaining bactericidal activity

Next steps

- Decipher what causes difference between agar and coupons and adjust plasma parameters to improve microbicidal efficiency
- Expand testing extremophilic organisms
- Selecting a plasma source configuration to move to the next TRL level that will allow us to test under normal and reduced gravity conditions

Testing relevant hardy and resistant organisms

- Collection of NASA cleanroom isolates
- including extremophiles
- radiation resistant species

Chelsi Cassilly, MSFC

- Testing whether bacteria can develop cold plasma resistance

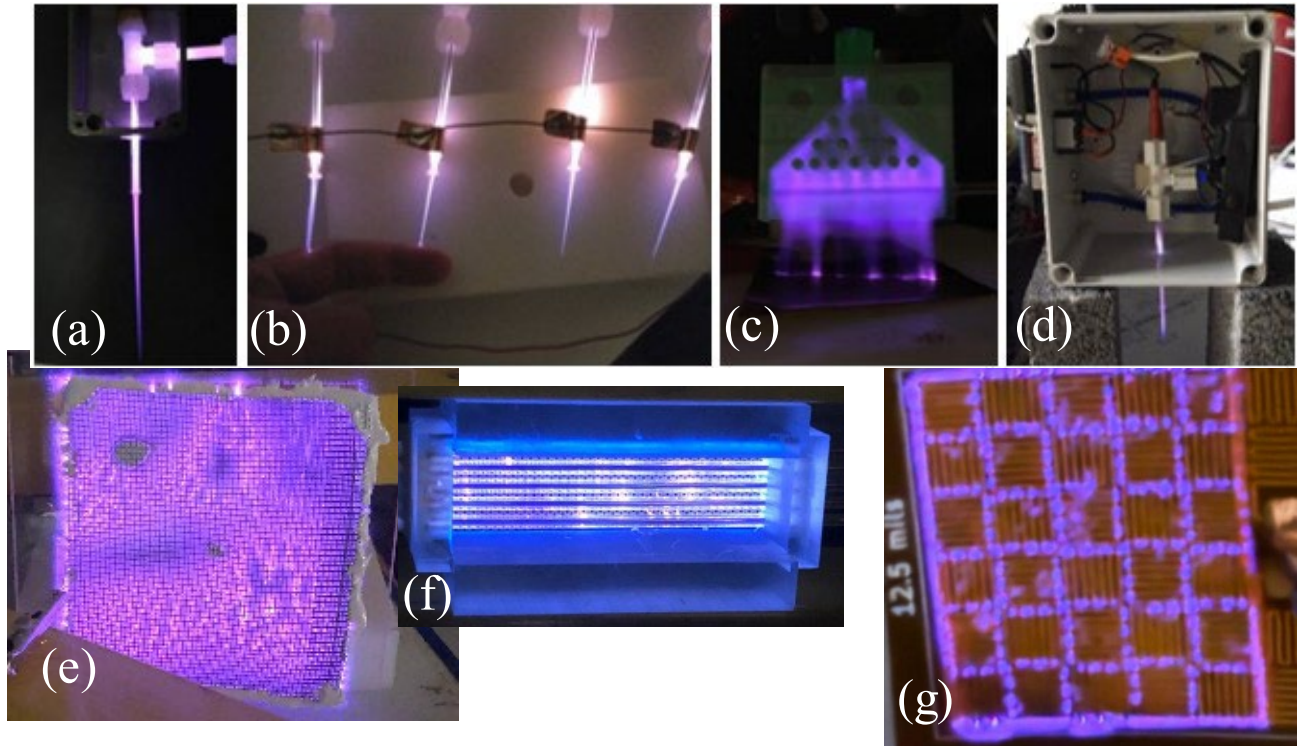
Carrie Waugh

Marina Ajinov

Selecting appropriate plasma source

- Pick geometry and type
- Tune operating parameters
- Portable device for flight

Gabe Xu, UAH

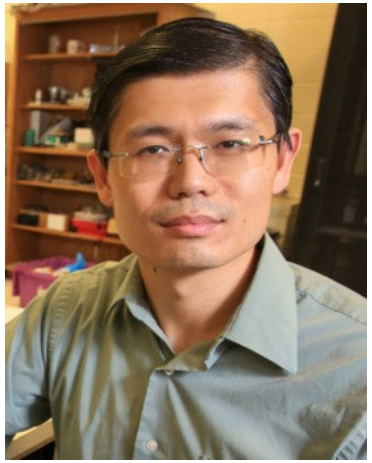


Examples of cold plasma sources developed at UAH
(a) a single APP jet
(b) 4 plasma jet array
(c) 2 inch wide plasma sheet
(d) portable plasma jet
(e)100 cm^2 surface DBD with air
(f)DBD plasma with air
(g) surface DBD generated in flexible polyamide sheet

Thanks to the Team!

John Mayo

Gabe Xu



Lanie Briggs

Chelsi Cassilly

Yo-Ann Velez Justiniano

Bhagirath Ghimire

Carrie Waugh

Marina Ajinov

Amy LeBleu DeBartola

Sysoeva Lab

Ryan Gott

UAH RCEU program; CPU2AL Seed grant (NSF EPSCoR)
NASA MSFC CAN Cooperative Agreement

Thank you!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Mechanism for cold plasma on dry coupons

- Cell state and/or Lack of water ?

Polycarbonate

Inconel 718

Yo Ann Velez Justiniano, MSFC

Non uniformity of cell distribution
DAPI stained bacteria on coupon

- MS analysis reactive species in situ using ACL capabilities

Ryan Gott, KSC

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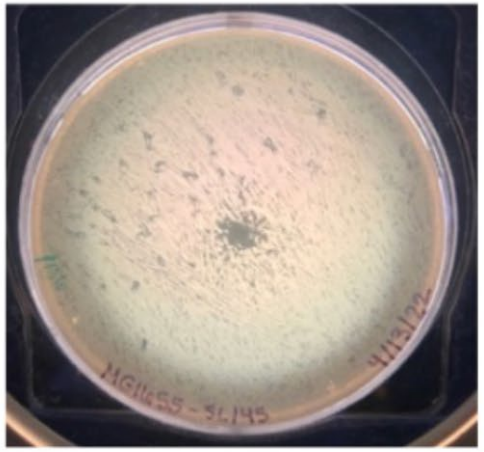
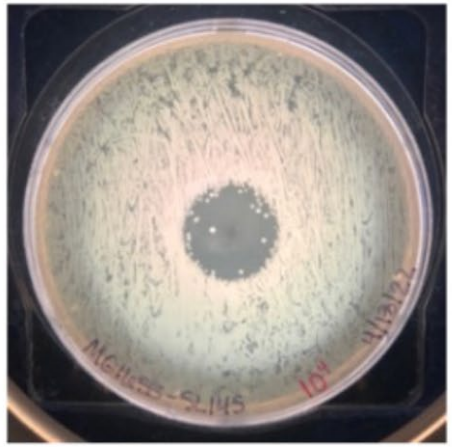
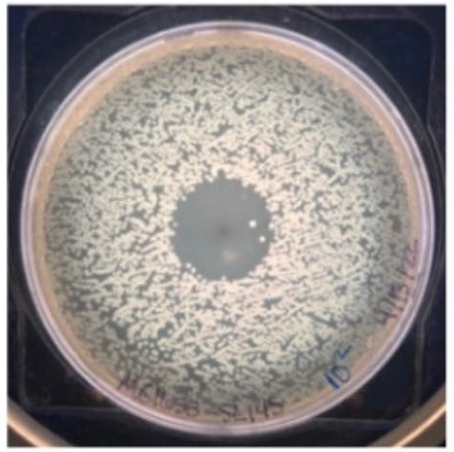


10^6 CFU/cm^2



10^4 CFU/cm^2



10^2 CFU/cm^2

2021 Seed grant from CPU2AL EPSCoR

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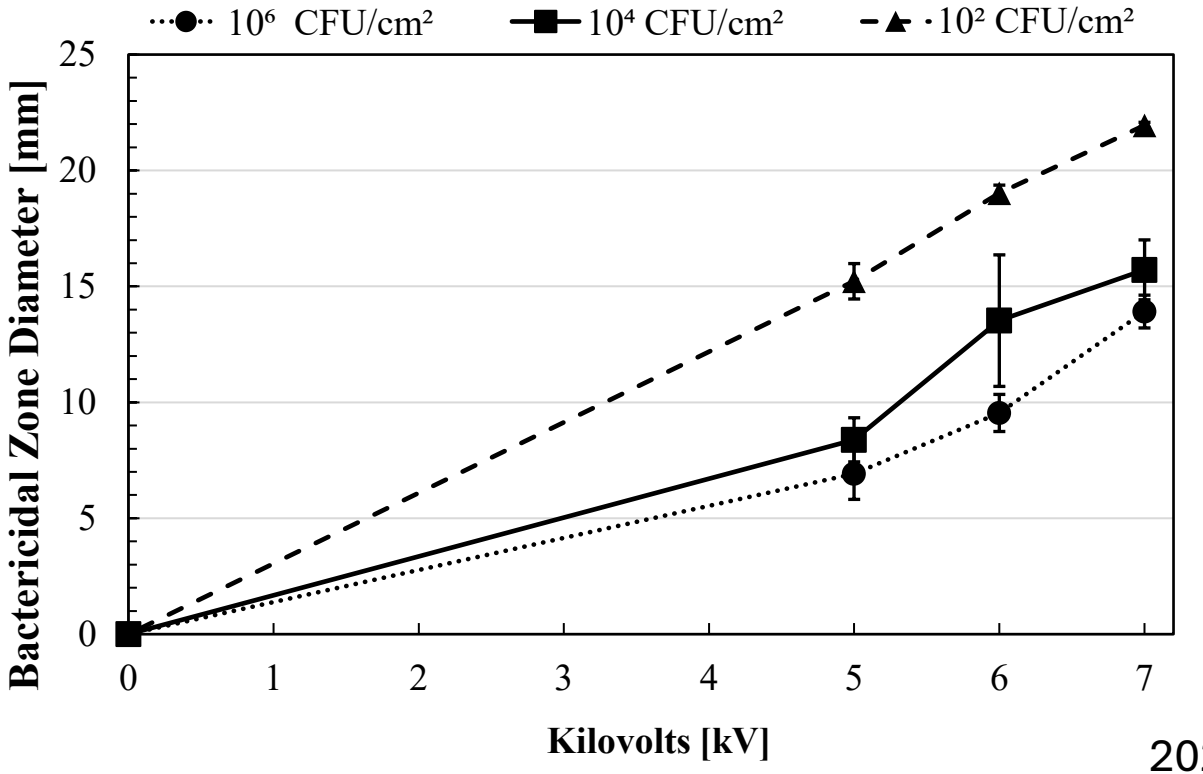

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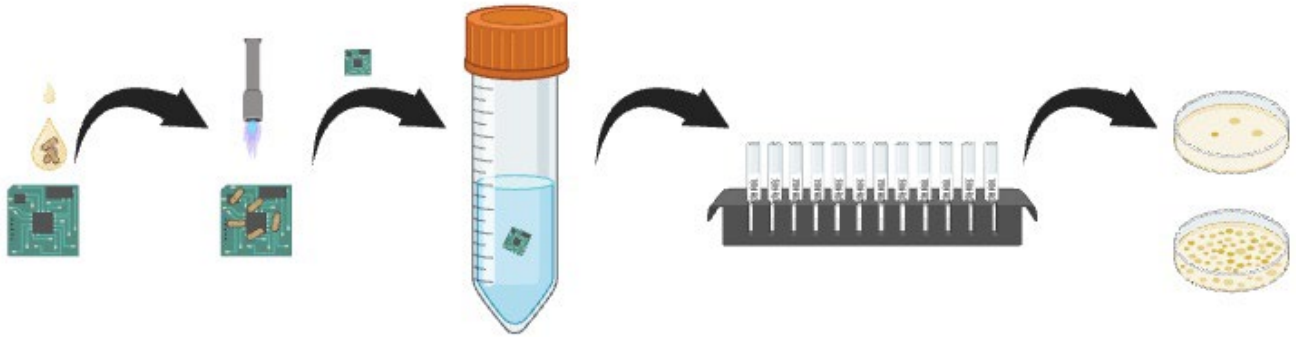
Quantification of bactericidal efficiency



BioRender

Deep space environment: friend or foe for PP?

Chelsi Cassilly, NASA MSFC

Atul Chander, NASA JPL

Deep space environment: friend or foe for planetary protection?

Objective 1: Quantify the Effects of Ionizing Radiation and Cryogenic Temperatures on Microbial Survival in Vacuum.

Objective 2: Characterize the Genotypes of Cleanroom Microorganisms and their Phenotypes towards Divergent Sterilization Processes.

Objective 3: Develop Genomics-lead Predictive Model to Assess Risk of Radiation Resistance.

Deep Space Environment: Friend or Foe for Planetary Protection?

Objective 1: Quantify the Effects of Ionizing Radiation and Cryogenic Temperatures on Microbial Survival in Vacuum. To measure the level of resistance against individual and combined space-related stressors, a radiation resistant cleanroom *A. koreensis* strain and a standard bioindicator *Deinococcus radiodurans* will be exposed to combinations of radiation, vacuum, and cryogenic temperature. We hypothesize that *A. koreensis* will demonstrate decreased survival when exposed to a combination of stressors since survival would likely require multiple mechanisms of resistance.

Objective 2: Characterize the Genotypes of Cleanroom Microorganisms and their Phenotypes towards Divergent Sterilization Processes. We will assemble complete genomes of all available cleanroom isolates using hybrid assembly of short and long read sequencing data. We will screen the cleanroom isolates collection for radiation resistance. Then we will test the cleanroom isolates for their survival against other sterilization regimes (alcohol, dry heat, cold plasma). We hypothesize that resistant isolates will have identifiable genetic determinants.

Objective 3: Develop Genomics-lead Predictive Model to Assess Risk of Radiation Resistance. Annotated genomes will be searched for known resistance-associated genes and used to identify new associated genes based on phenotypic data. A presence-absence matrix of genes will be used to construct machine-learning models to train on genomes of radiation-resistant and -sensitive organisms. The optimally performing model will be further tested and validated to ultimately predict/classify radiation resistance of novel microorganisms.

Genomics-lead predictive model to assess risk of radiation resistance