Technical Meeting on Emerging Applications of Plasma Science and Technology on 19–22 September 2023, Vienna, Austria

Atmospheric Pressure Plasma jet for Biomedical Applications

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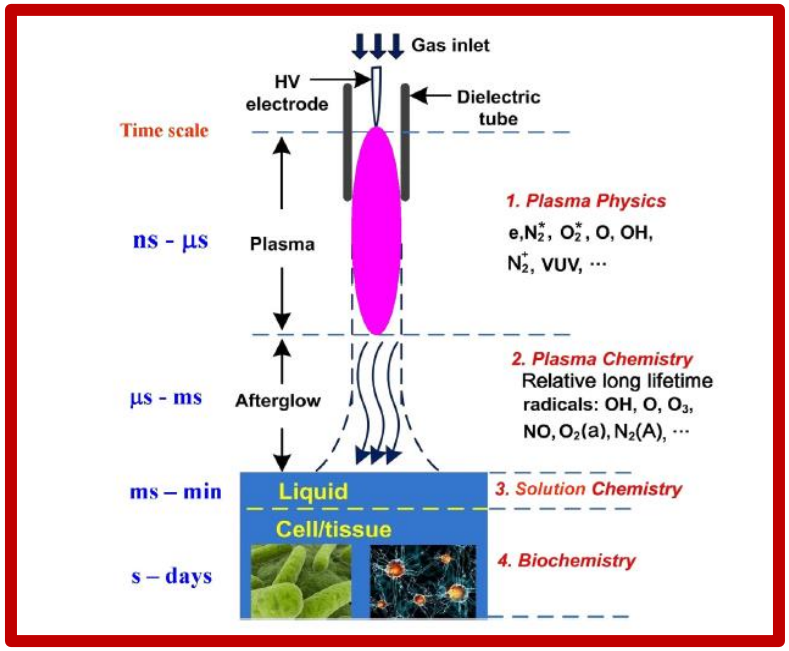
प्लाज़्मा अनुसंधान संस्थान
Institute for Plasma Research

Content

- Introduction of Atmospheric Pressure Plasma Jet (APPJ)
- APPJ at Institute for Plasma Research (IPR), India
  - Characterisation of APPJ
- APPJ for biomedical applications
  - Cell death of cancerous cells
  - Treatment of fungal skin infections
  - Hand sanitization
- Air Plasma array for Sterilisation of surfaces
- APPJ treated water and its biomedical application
- Conclusion

Atmospheric Pressure Plasma Jet



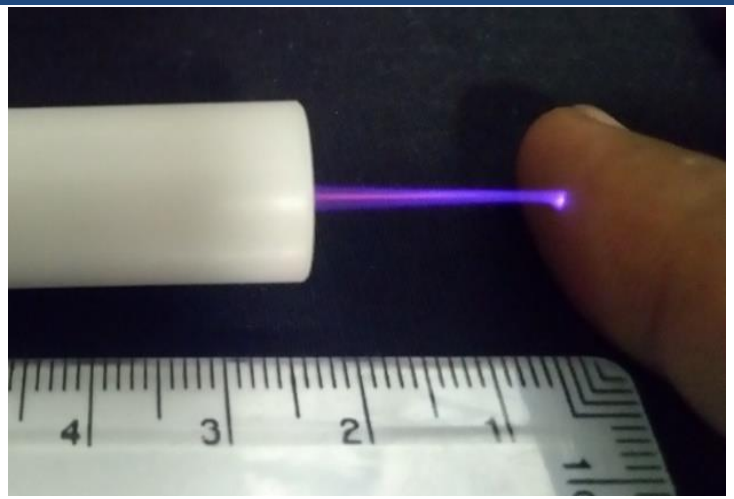
Lu et. al, Physics Reports 630 (2016) 1–84

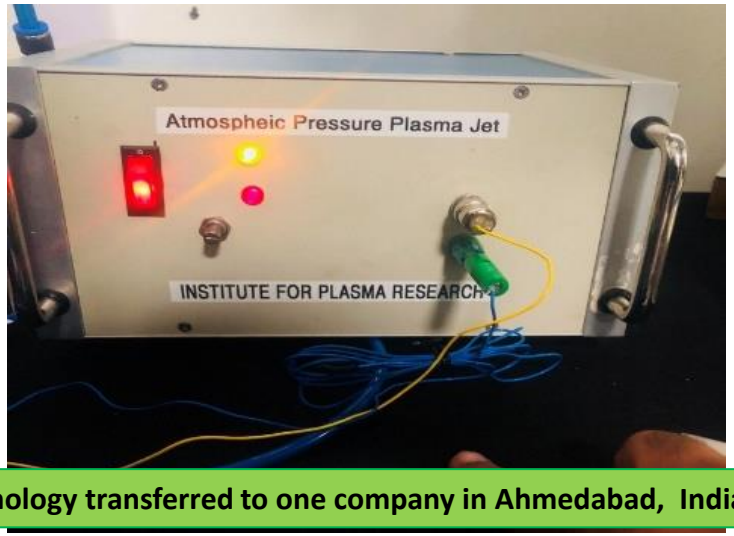
- ❑ Non thermal
- ❑ Glow discharge like plasma
- ❑ Atmospheric pressure
- ❑ Electrons, ions, UV and reactive species
- ❑ Overall Gas temperature cold

Biomedical Applications

- ❖ Wound healing
- ❖ Removal of Skin infection/black spots from skin.
- ❖ Sterilization of skin such as hands.
- ❖ Dentistry for teeth bleaching and sterilization during root canal treatment.
- ❖ Cell death of cancerous cells without affecting the healthy tissues.

Atmospheric pressure Plasma jet (APPJ) @ Institute for Plasma Research





Technology transferred to one company in Ahmedabad, India

This plasma Jet is developed by IPR for medical application

It is cold touchable plasma jet using Dielectric barrier discharge

It has working gas as Helium, Argon

It operates on high frequency power supply

Power few watt.

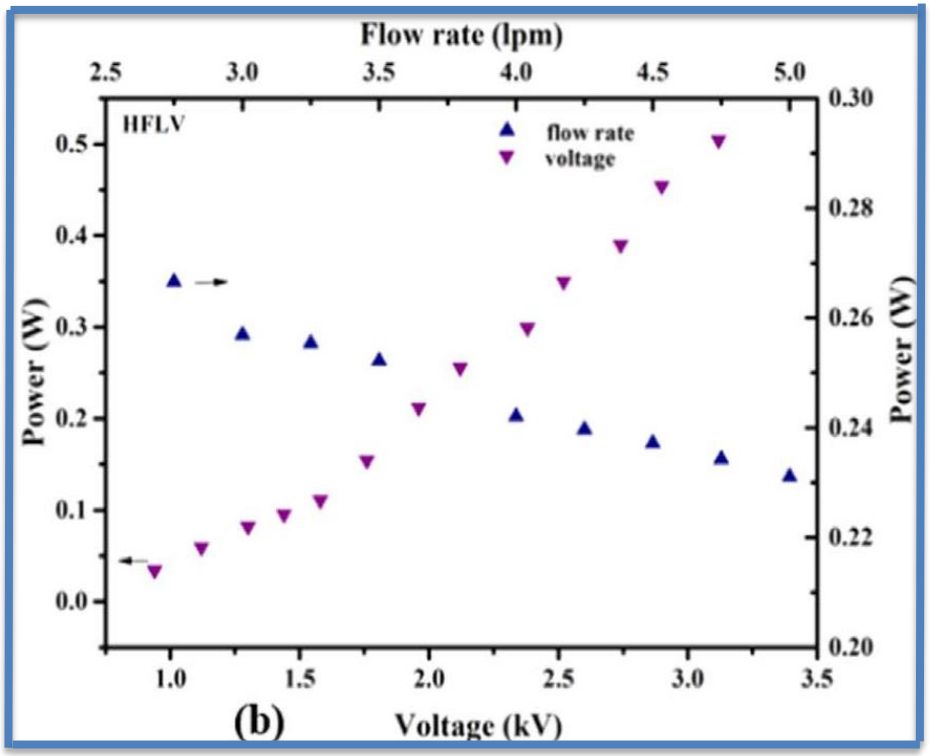
It has gas flow rate is 2 -5 lpm and temperature around 40 degree Celsius

A Vaid, C Patil, A Sanghariyat, R Rane, A Visani, S Mukherjee, Alphonsa Joseph et al ,Neurology India | Volume 68 | Issue 1 | 2020 and

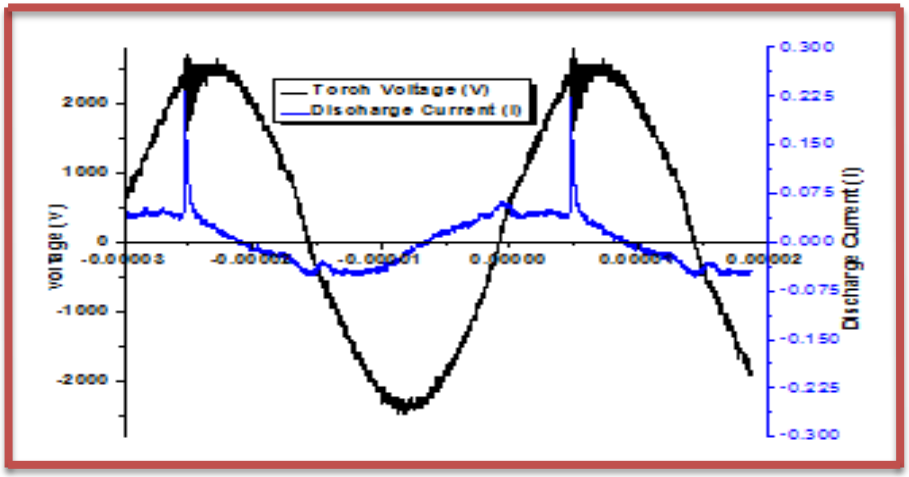
Indian Patent application no. 3727/MUM/2015

Characterization of APPJ

Power measurement



Power



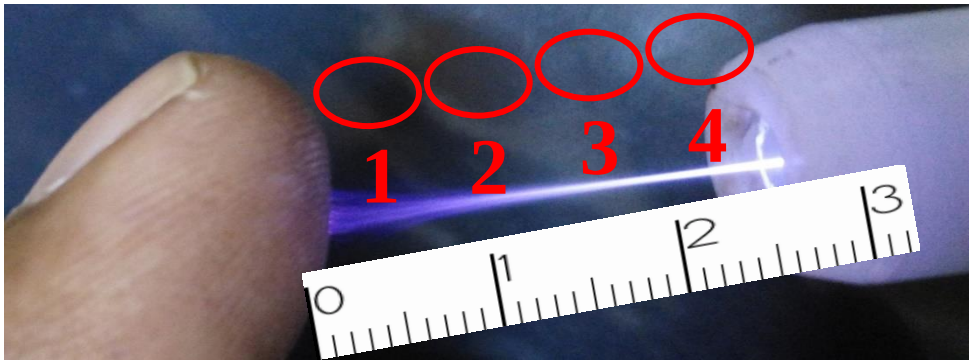
I-V characteristics of AAPJ demonstrating diffuse discharge instead of filamentary discharge.

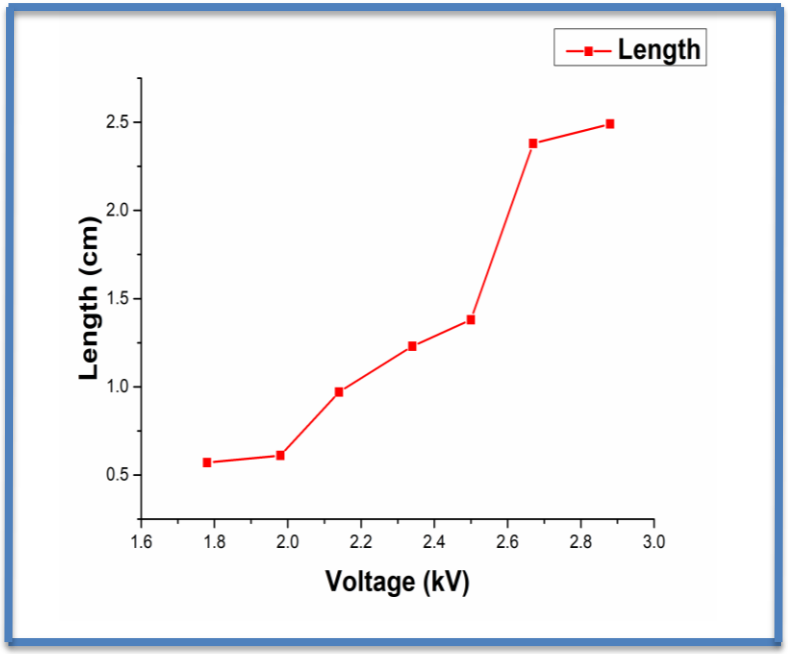
Discharge power increases with voltage

Characterization of APPJ

Plume Length measurement

Plasma Plume Length





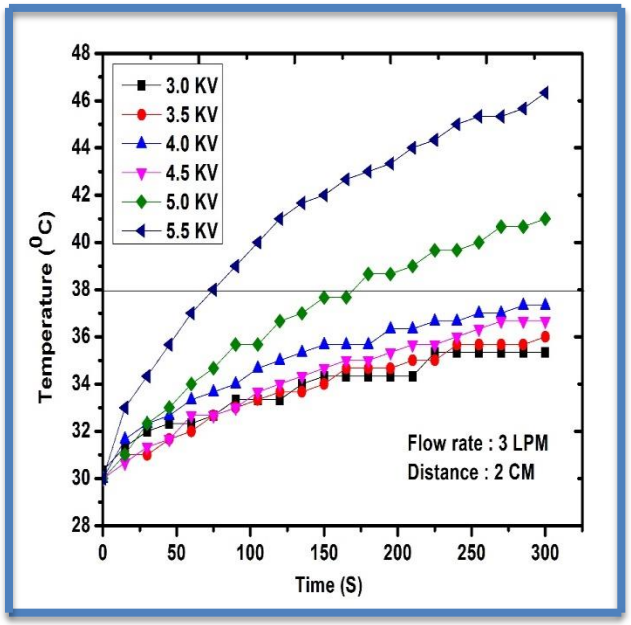
1. Bottom plume, 2. Lower of middle plume
3. Middle plume, 4. Upper plume

Plume length increases with voltage having plume length of 2.5 cm at 3 KV

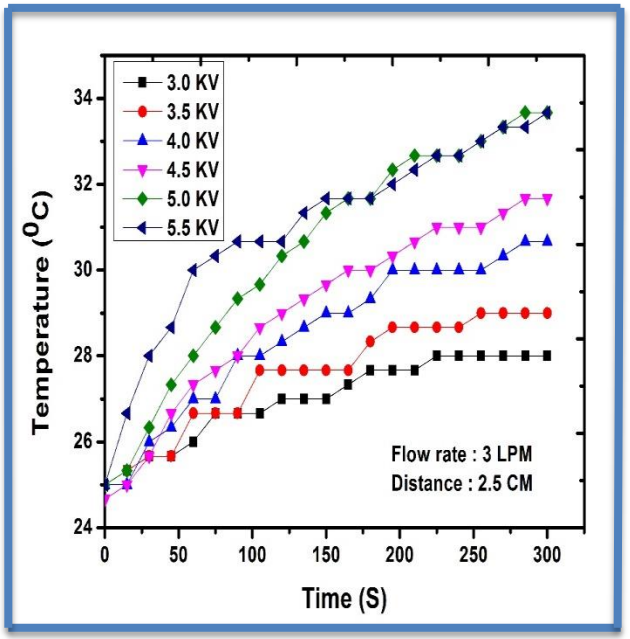
Characterization of APPJ

Plume Temperature measurement

At distance of 2 cm from plume exit



At distance of 2.5 cm from plume exit

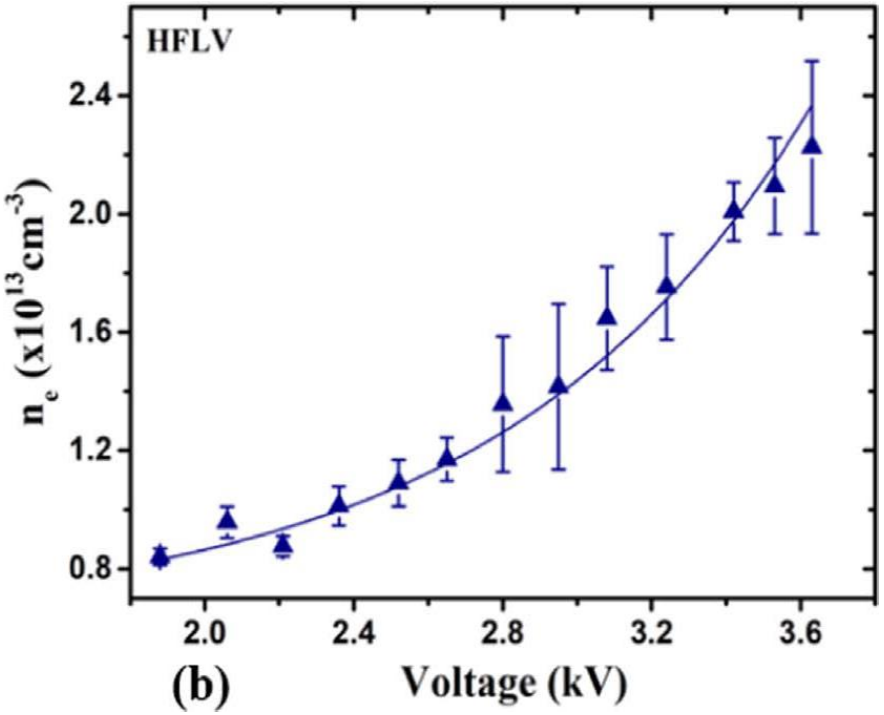


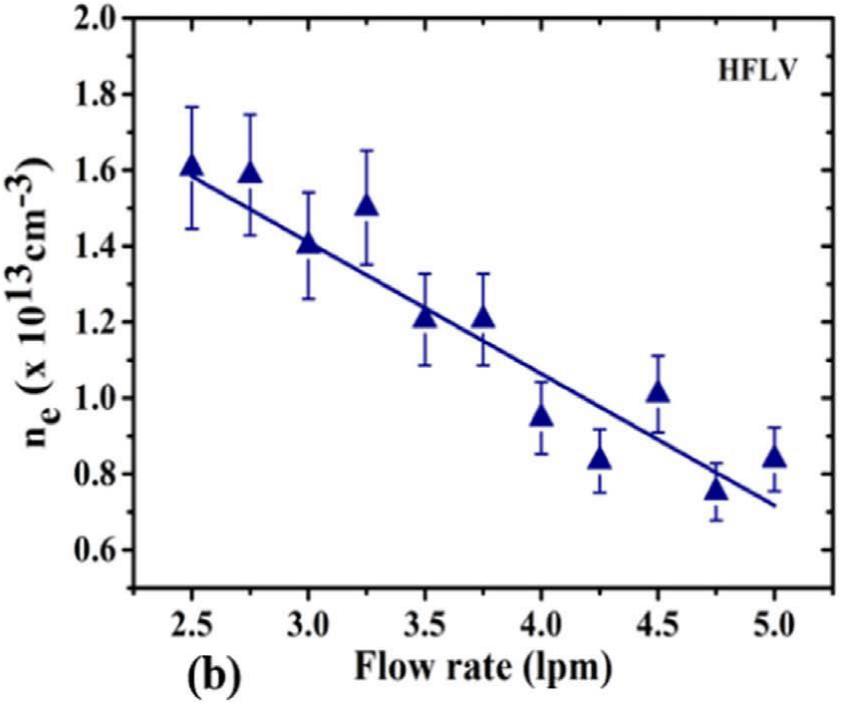
Helium Gas plasma jet

Temperature measured at the tip of the plume

Temperature of the plume remains below 40^0 C

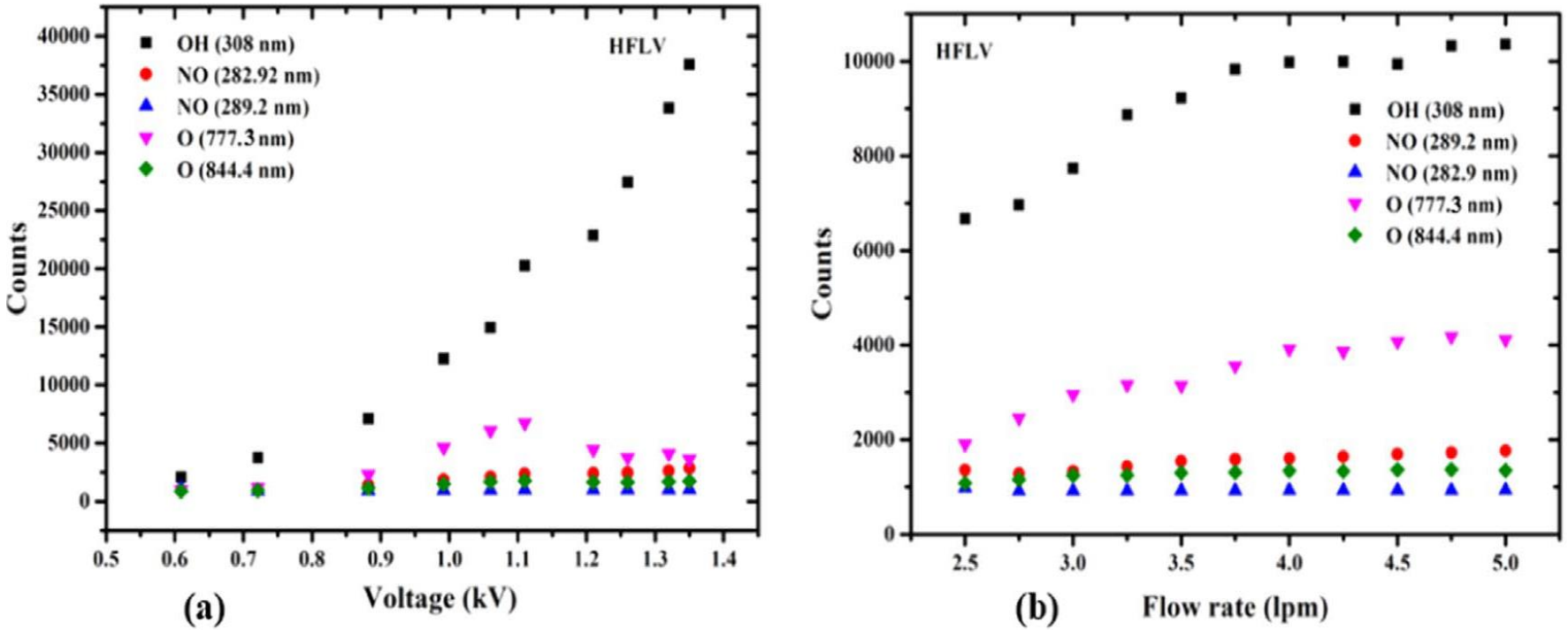
Plasma Density measurement using Optical Emission Spectroscopy





Plasma density increases with voltage while it decreases with helium gas flow rate

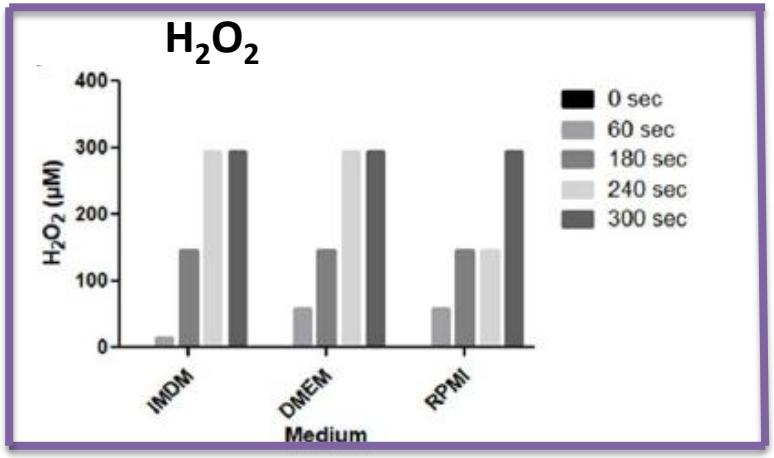
Reactive Species measurement

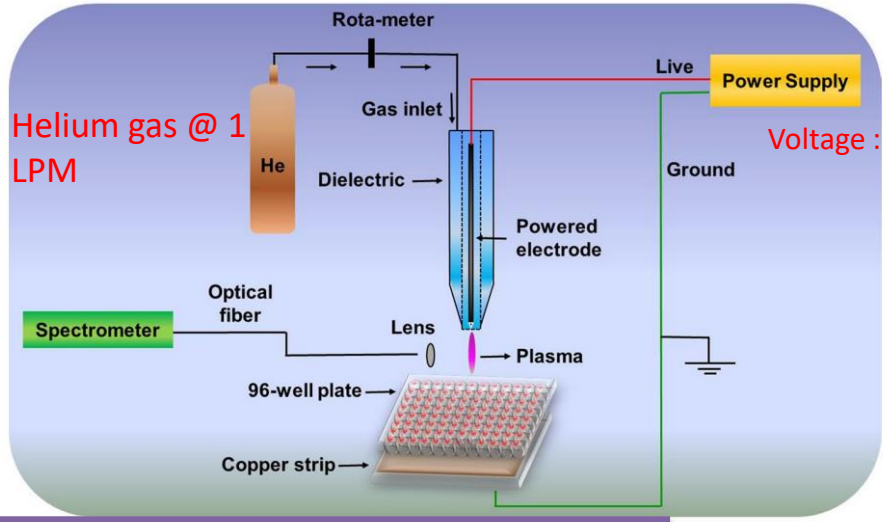


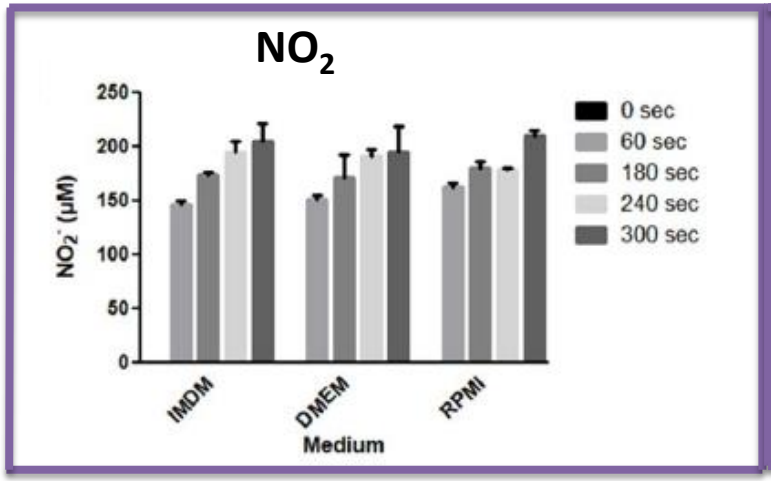
Species measurement : Optical Emission Spectroscopy

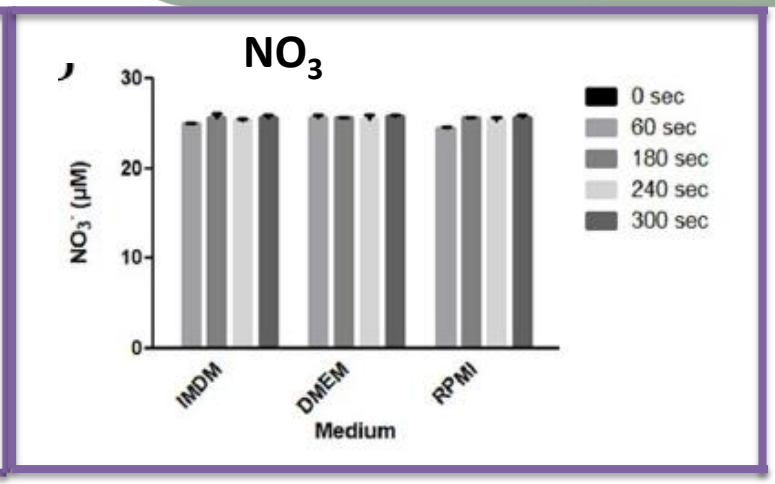
OH and O are greatly influenced by voltage and flow rate

ROS and RNS Measurement in Plasma treated Cell Culture media









DMEM: Dulbecco's Modified Eagle Medium
RPMI: Roswell Park Memorial Institute
IMDM: Iscove's Modified Dulbecco's Med**ium**

Biomedical Applications of APPJ explored at Institute for Plasma Research

Applications

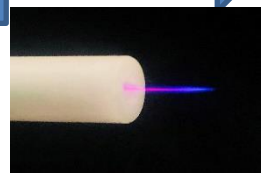
- **Cell death of cancerous cells :** In vitro and in Vivo study
- **Treatment of fungal skin infections :** In vivo study
- **Hand sterilization :** In vitro and in Vivo study
- **Air Plasma array for Surface Sterilization**
- **APPJ treated water for biomedical application**

In-vitro studies for cancer Cell lines

The current treatment modality of oral cancers is surgical resection of the tumor in conjunction with chemotherapy or radiation therapy

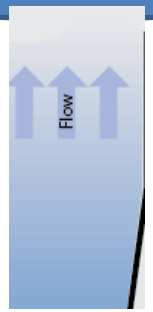
Ionizing Radiation (0,2,4,6,8 Gy)

Helium APPJ (0,60,180,240,300 Second)



ITOC-03 (Gingivo buccal squamous cell carcinoma),
MCF7 (breast adenocarcinoma cell line)
HEK293 (human embryonic kidney cell line)

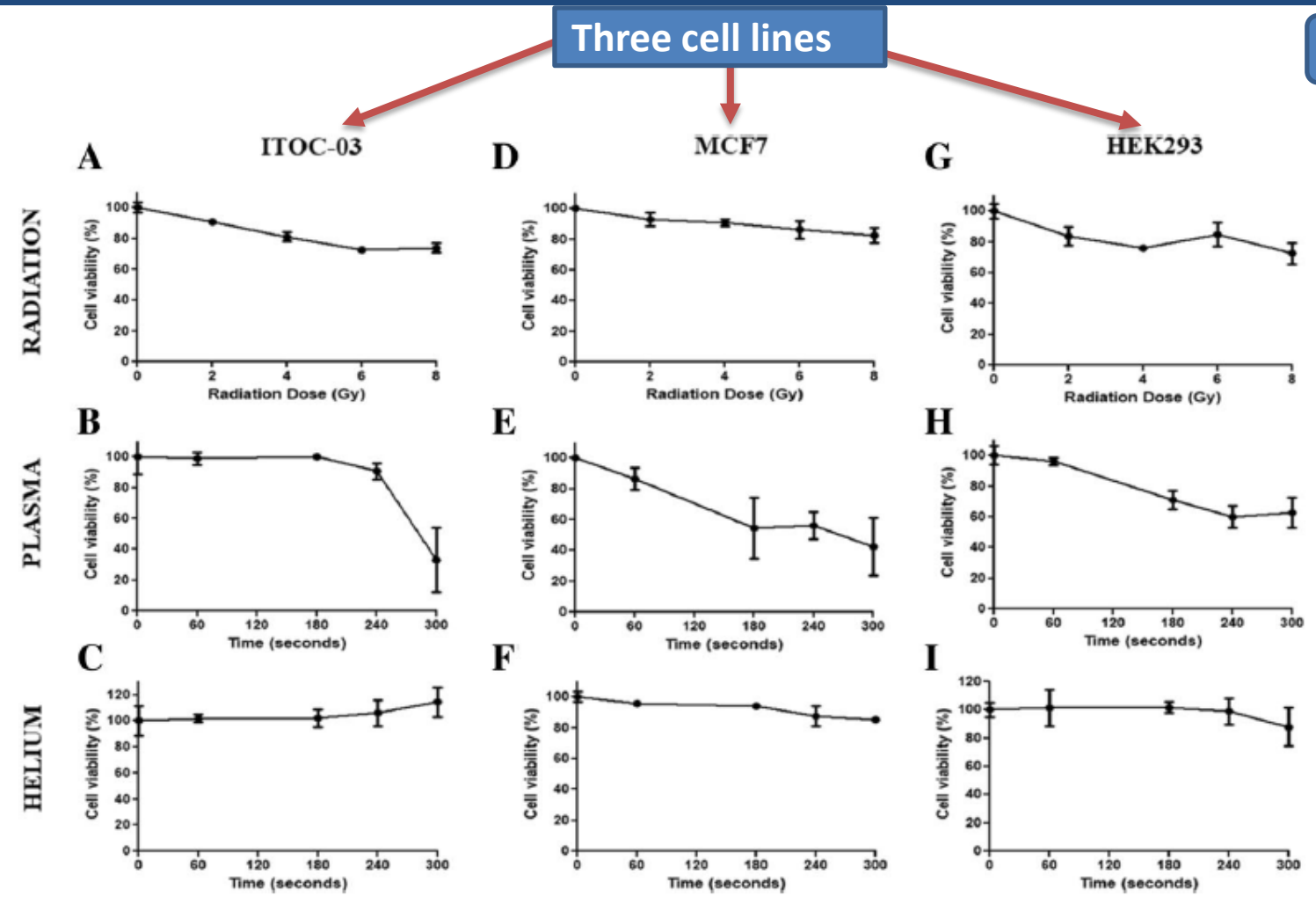
Helium Gas (0,60,180,240,300 Second)



Limitations of the above are requires post-surgery sometimes, high radiation dose in radiotherapy and chemo-resistance in chemotherapy

Cell Viability ?

Comparative Cell viability for Radiation, Helium plasma and Helium gas treatment



Cell Viability

70-80 % for radiation

20-30 % for Helium plasma jet

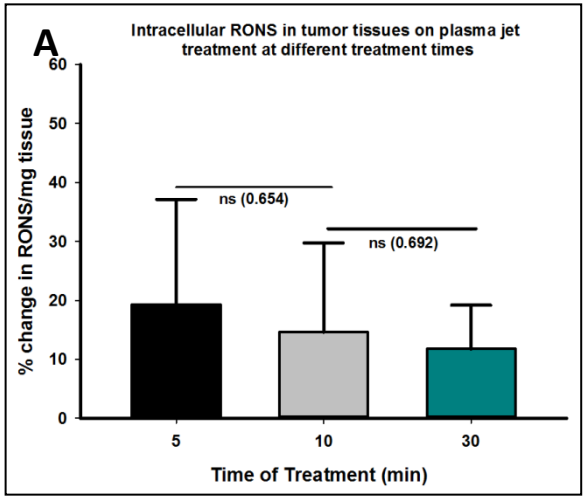
No effect

Kshama Pansare, Akshay Vaid, Saurav Raj Singh, Ramkrishna Rane,Anand Visani,Mukesh Ranjan, C. Murali Krishna, Rajiv Sarin, Alphonsa Joseph
Plasma Chemistry and Plasma Processing, **42**, 163–178 (2022)

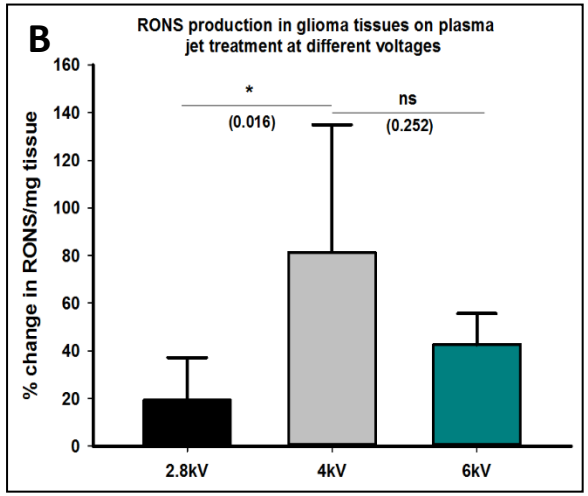
Effectiveness of APPJ in Human brain tumor tissues resected from glioma patients

The major obstacle in treating high-grade gliomas is the presence of residual cancer cells at the tumor margins post resection.

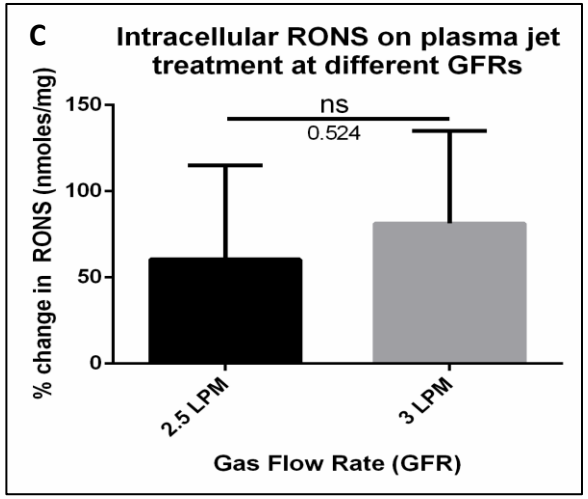
Helium APPJ was applied on resected tumor specimens obtained from patients (~ 30 patients) with glioma at AIMS, Delhi .



Exposure time



Voltage



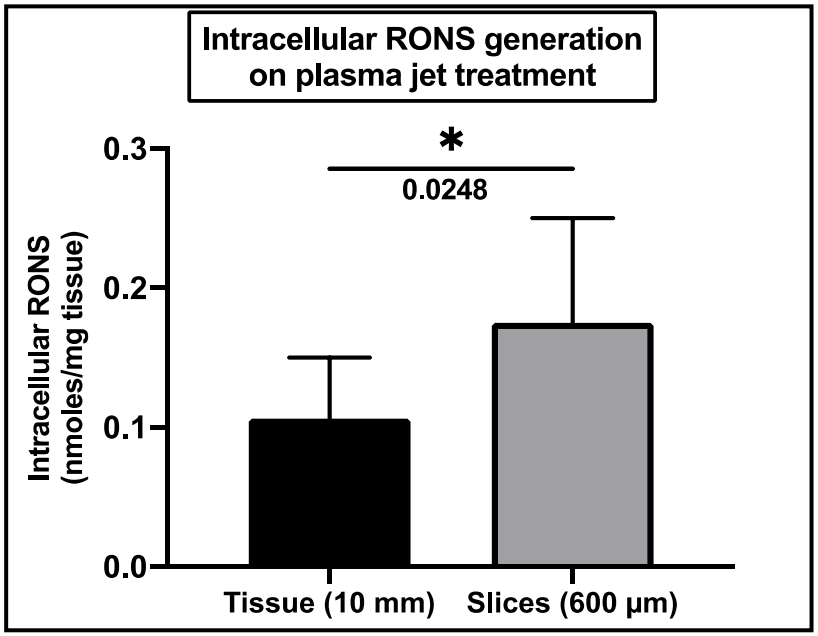
Gas Flow rate

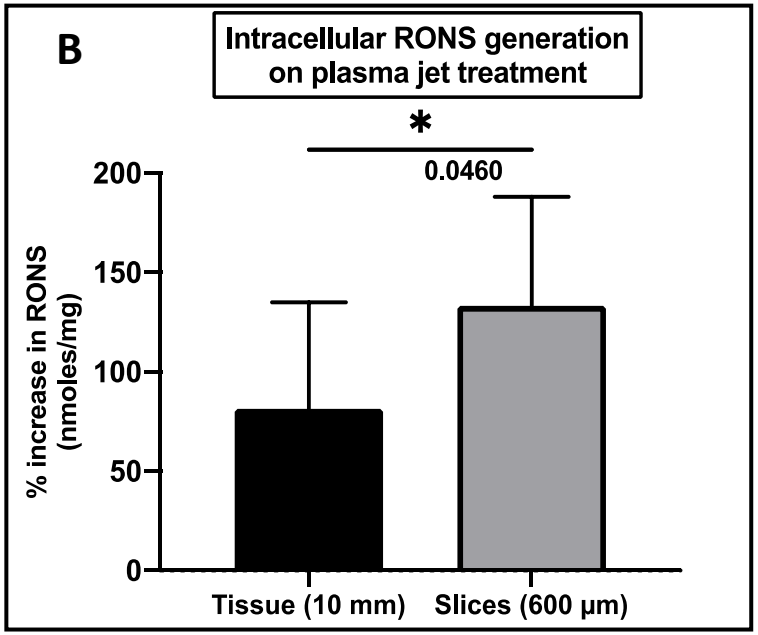
The results show that CAP was effective in generating enhanced levels of RONS in glioma samples

The study was carried out in accordance with the recommendations of the institutional ethics committee (IEC) of All India Institute of Medical Sciences (AIIMS), New Delhi, India (#IEC-535/03.11.2017, RP-10/2017

Cold Atmospheric Plasma (CAP) treatment increased Reactive Oxygen and Nitrogen Species (RONS) levels in tumor samples obtained from patients with low-grade glioma, submitted to Biomedical Physics & Engineering Express.

Effect of Slice Thickens on RONS generation

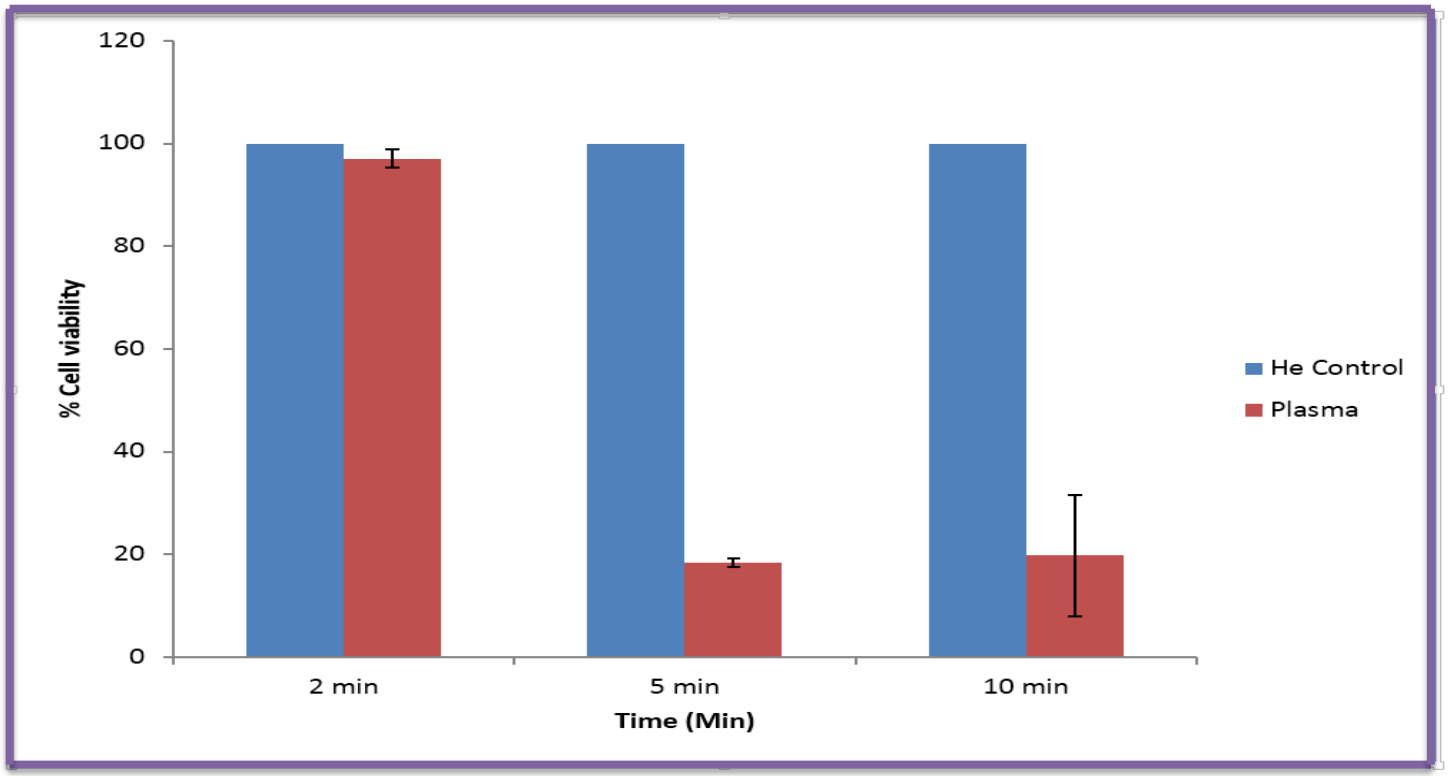




APPJ jet is more effective in thinner samples as compared to thick tumor samples for RONS generation

Treatment of Lung cancer cells by plasma jet (In-vitro Studies)

Atmospheric pressure plasma jet interaction with A549 cells (lung cancer cell lines)



It is observed that after 5 min of treatment cell viability goes down to 20 %

Treatment of fungal skin infections : In vivo study

Skin treatment (Tinea Cruris-fungal infection)

Fungal Infection at hand

Fungal Infection at foot

Argon plasma jet @ 3 LPM flow rate

Treatment involves

Appropriate antifungal ointment and tablets

Skin treatment (Tinea Cruris-fungal infection)

Initial infection

First week treatment

second week treatment

Treatment time = 10 min

Third week treatment

Treatment time = 10 min

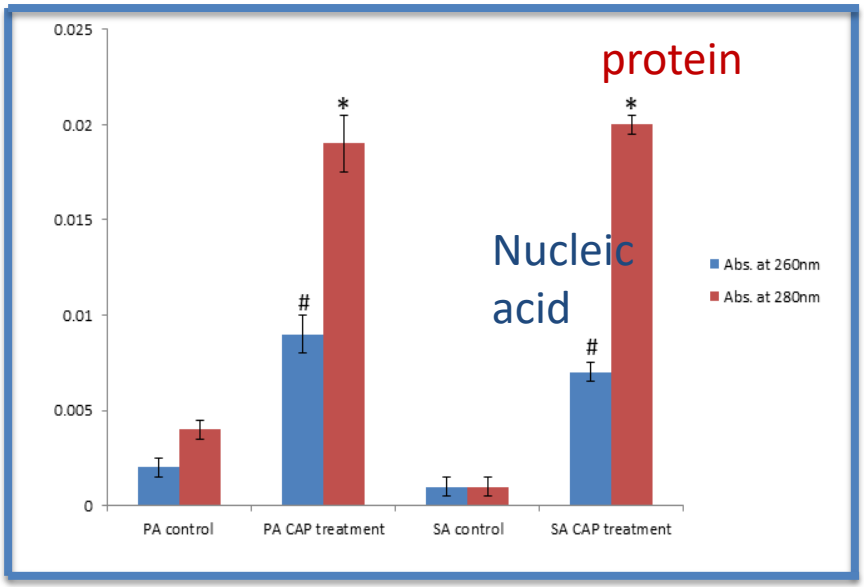
Experiments done at PG Medical college, Kolkota, dermatology department by our collaborator Dr. Abhijit Majumdar

Hand Sanitization by APPJ : In vitro and in Vivo study

Disinfecting effects of He-CAP was evaluated on two bacterial strains namely: gram positive *Staphylococcus aureus (SA)* and gram negative *Pseudomonas aeruginosa (PA)*

Bacterial strains were isolated from finger dabs of volunteers. Helium APPJ was applied for 05 minute exposure to the bacterial culture

Nucleic acid (DNA) and Protein concentrations of *S. Aureus* and *P. Aeruginosa*



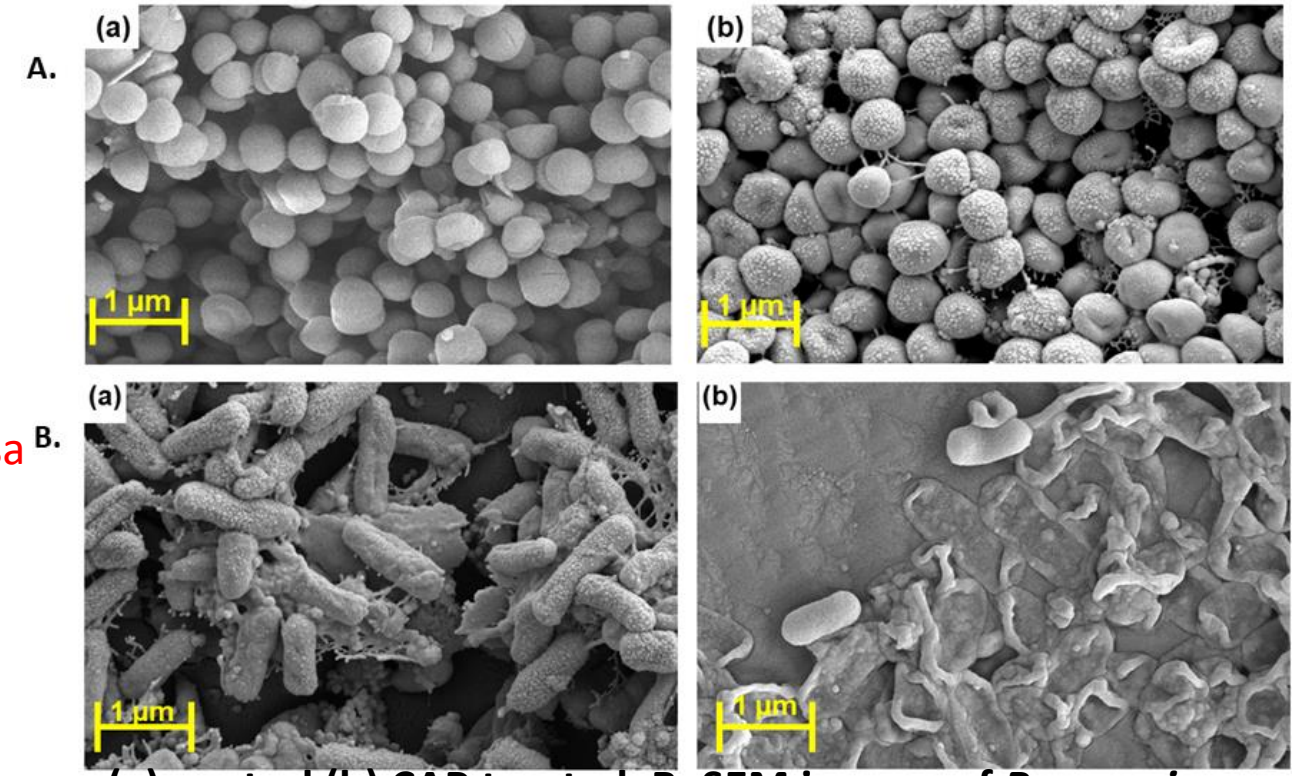
* represents significant differences in protein concentration between control and treatment groups. # represents significant difference in nucleic acids content between control and treatment groups

SEM images of bacteria after plasma treatment

control Plasma treated

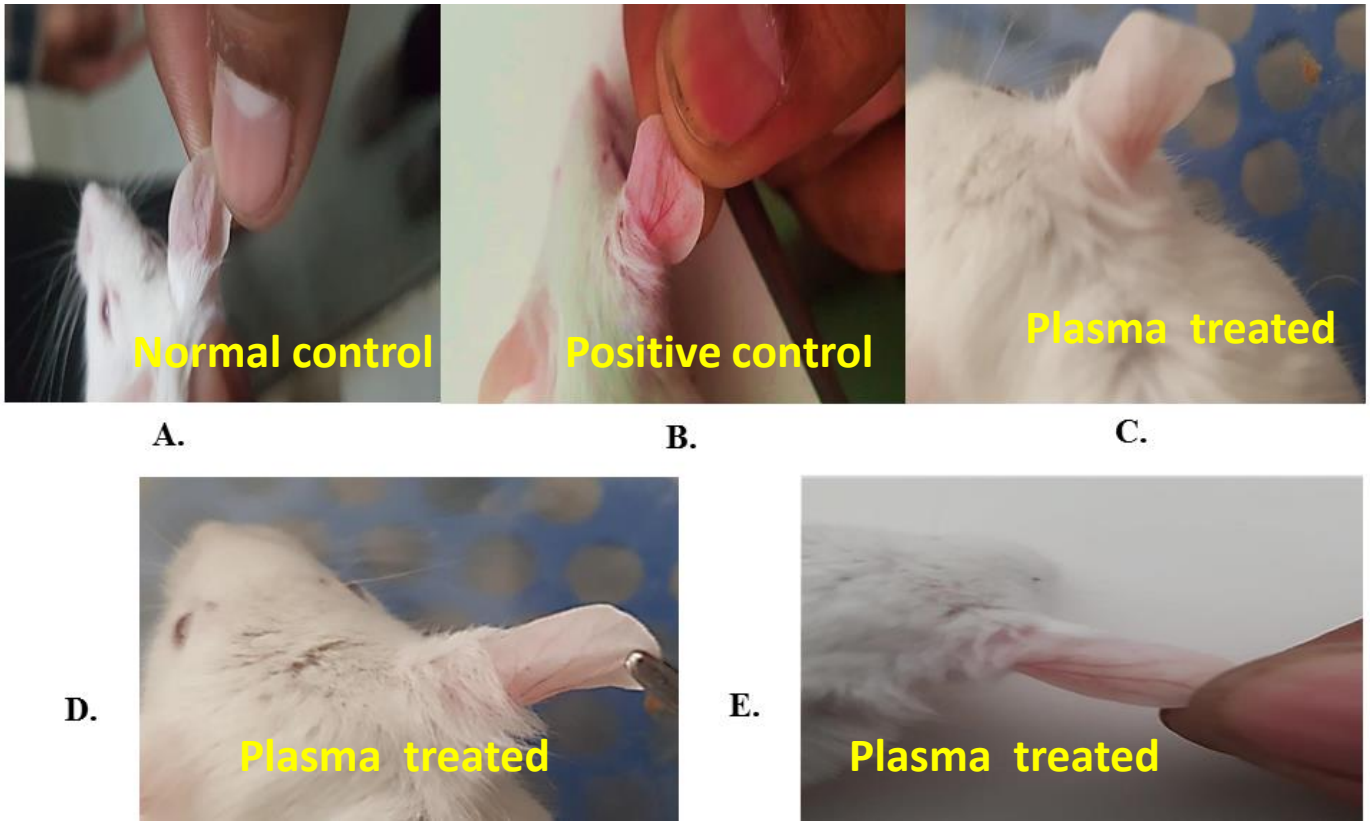
Staphylococcus aureus Bacteria

Pseudomonas aeruginosa Bacteria



A. SEM images of *S. aureus*; (a) control (b) CAP treated. B. SEM images of *P. aeruginosa*; (a) control (b) CAP treated. Remarkable differences in the morphology of both the bacteria following CAP exposure are visible to that of control group. CAP treatment resulted in morphological disruptions.

Sensitization evaluation of APPJ treatment



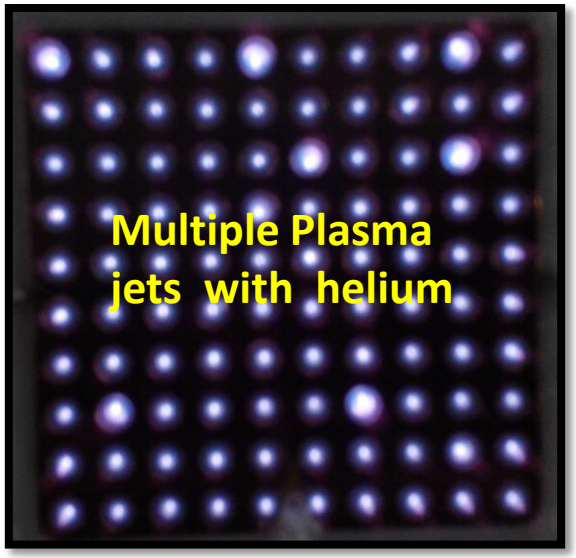
Swiss albino mice were selected for the study.

No groups except positive control (croton oil treated) showed edema or erythema of any grade. Erythema was witnessed in positive control (B). Images taken on last day (day 6).

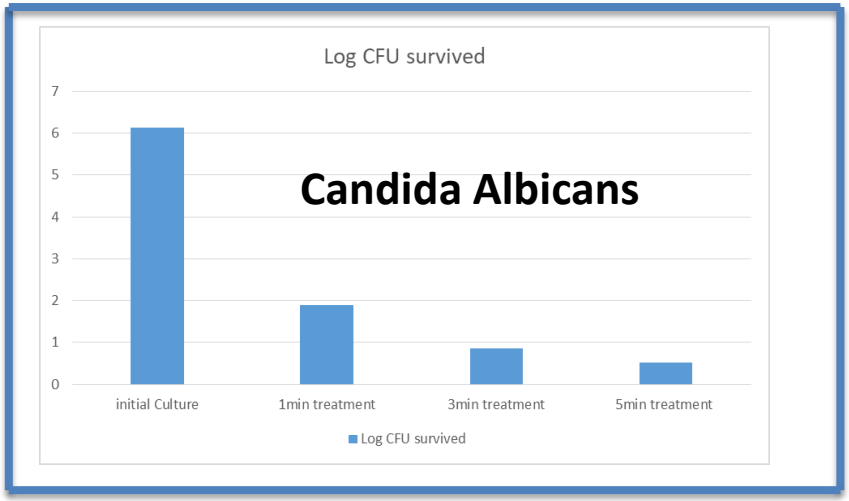
Representative images of ear lobes of mice treated with local irritant (croton oil) and cold atmospheric plasma (CAP). A. Normal control B. Positive control C. D1 D. D2 and E. D3.

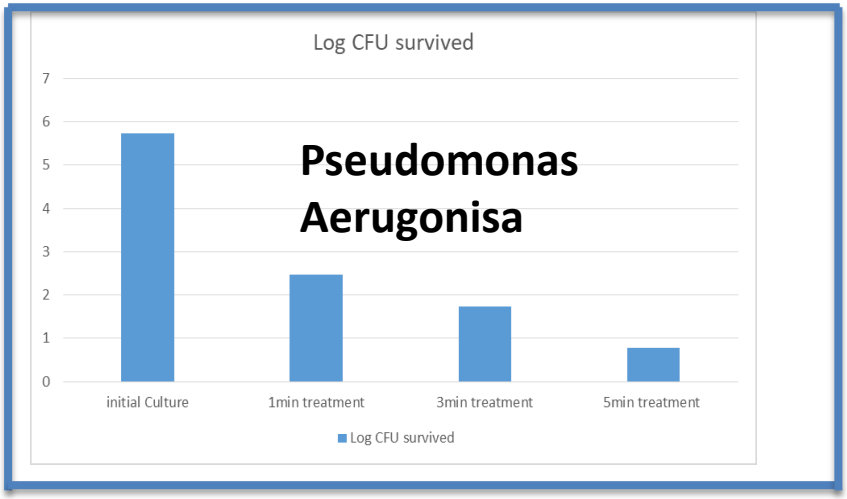
Protocol of the proposed study was presented to animal ethics committee and approval was taken (Approval no. IP/PCOL/FAC/27/2020/ 015).

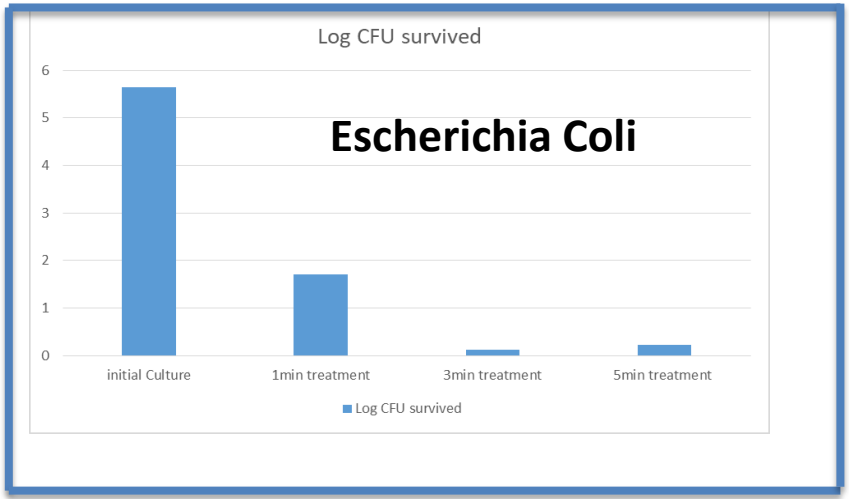
Some Other APPJ at IPR

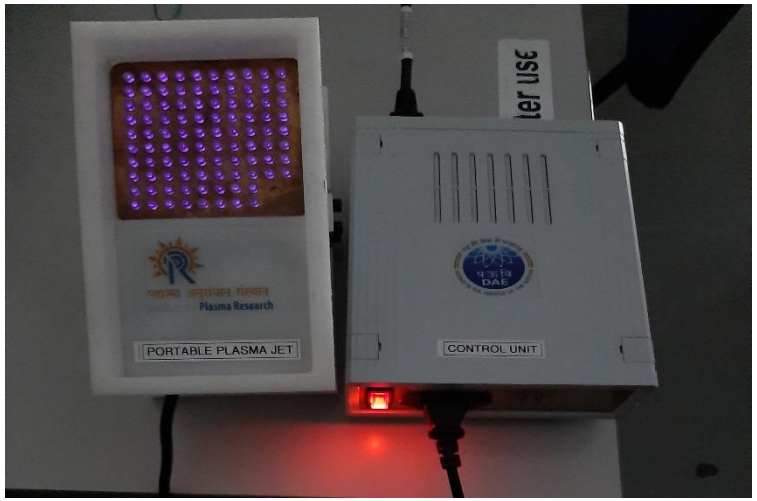


Antifungal and Antibacterial study Of micro plasma array using air

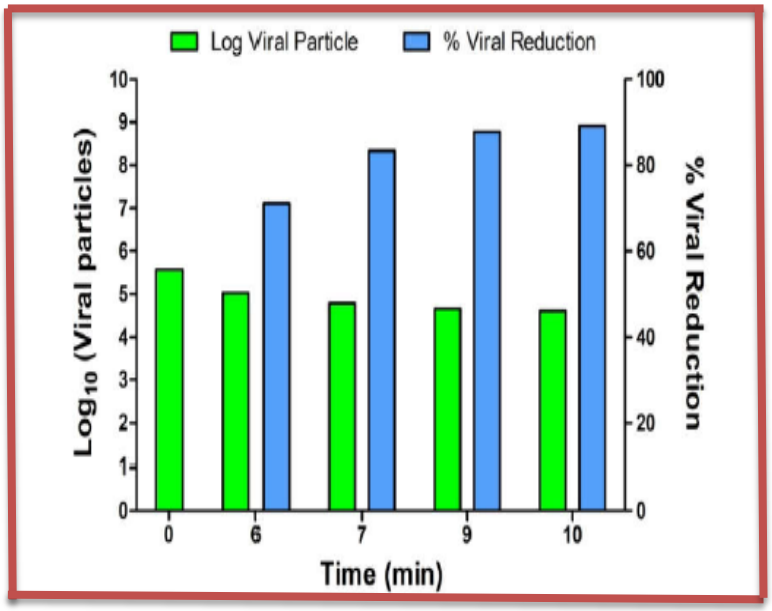


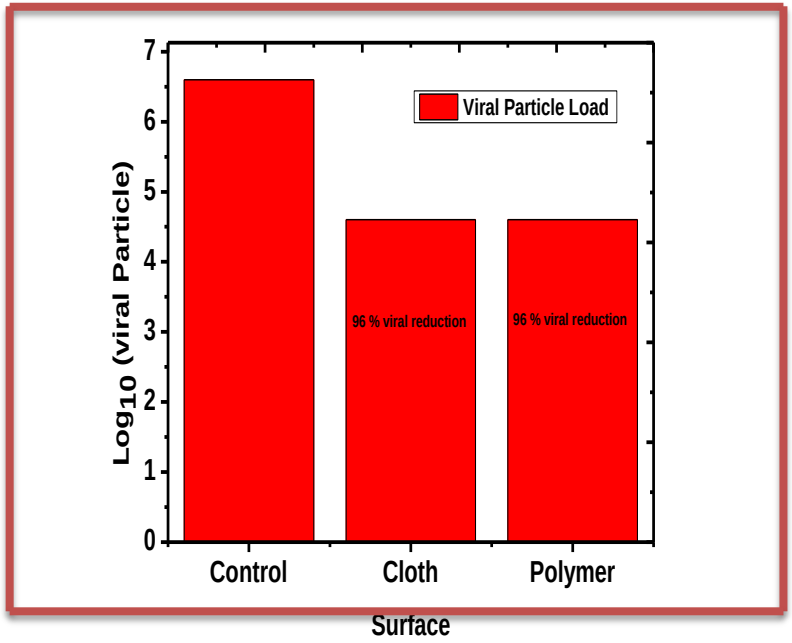






Antiviral study of micro plasma array using air for SARS-CoV-2 virus



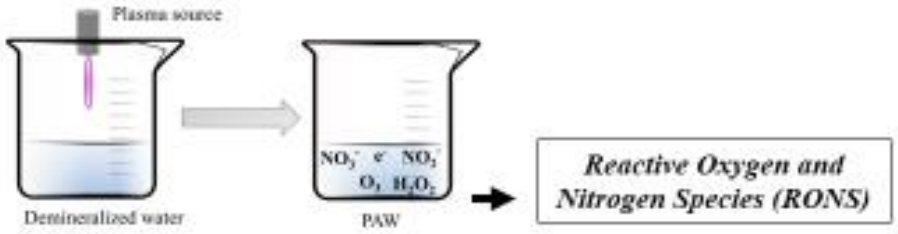


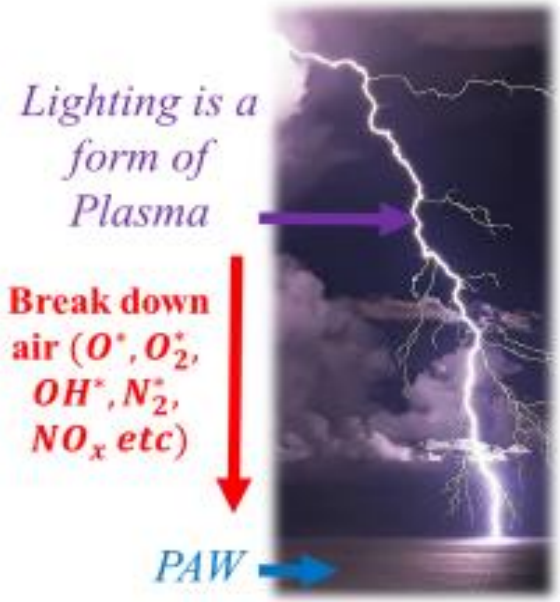
- The culture of SARS-CoV-2 virus was treated for different time scales.
- viral RNA was extracted from the sample by using a viral/pathogen extraction .
- Then a qRT-PCR method was used to determine the Ct value

After plasma treatment of more than 5 minutes the viral load reduces to ~ 90%.

APPJ treated water : Plasma activated water (PAW)

Plasma Activated Water and its Potential Applications as Antimicrobial & Anti-fungal Reagent



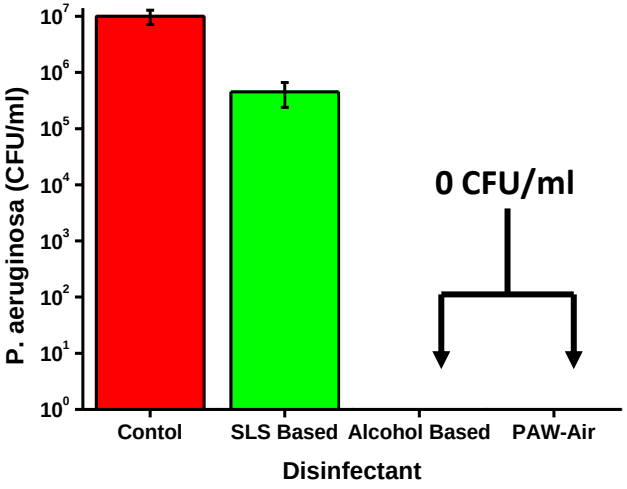


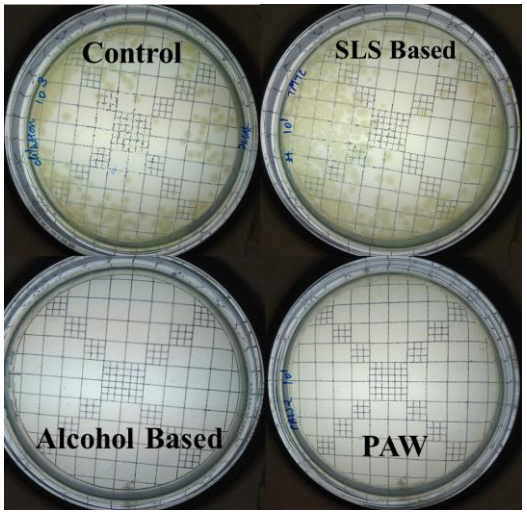
Natural generation of PAW

Experimental setup of PAW

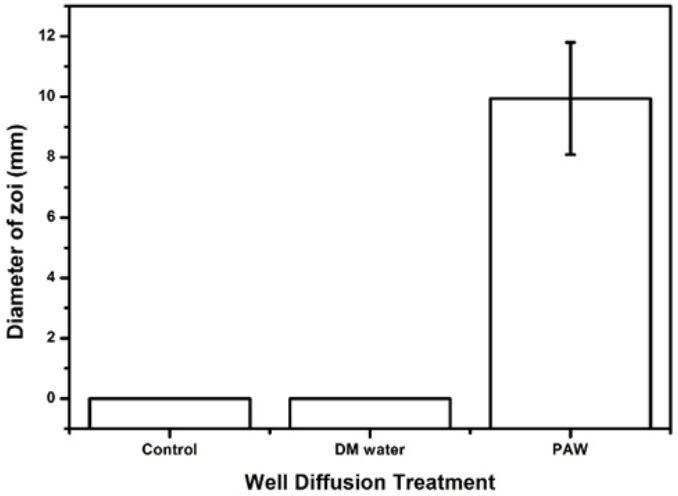
PAW inactivation study on *P. aeruginosa*

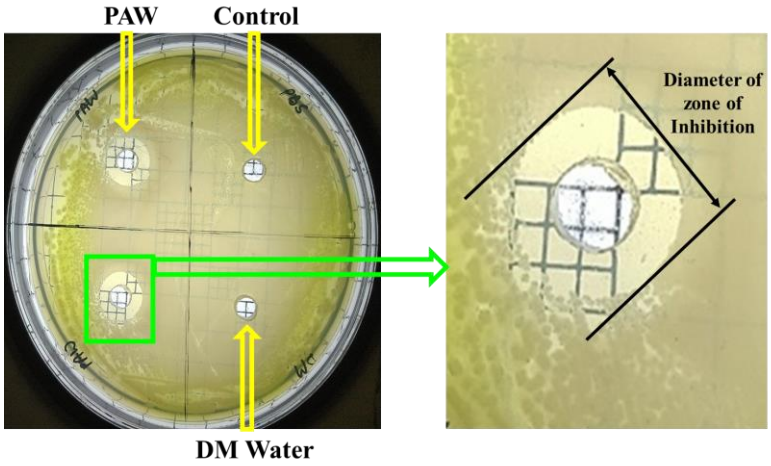
Spread Plate method





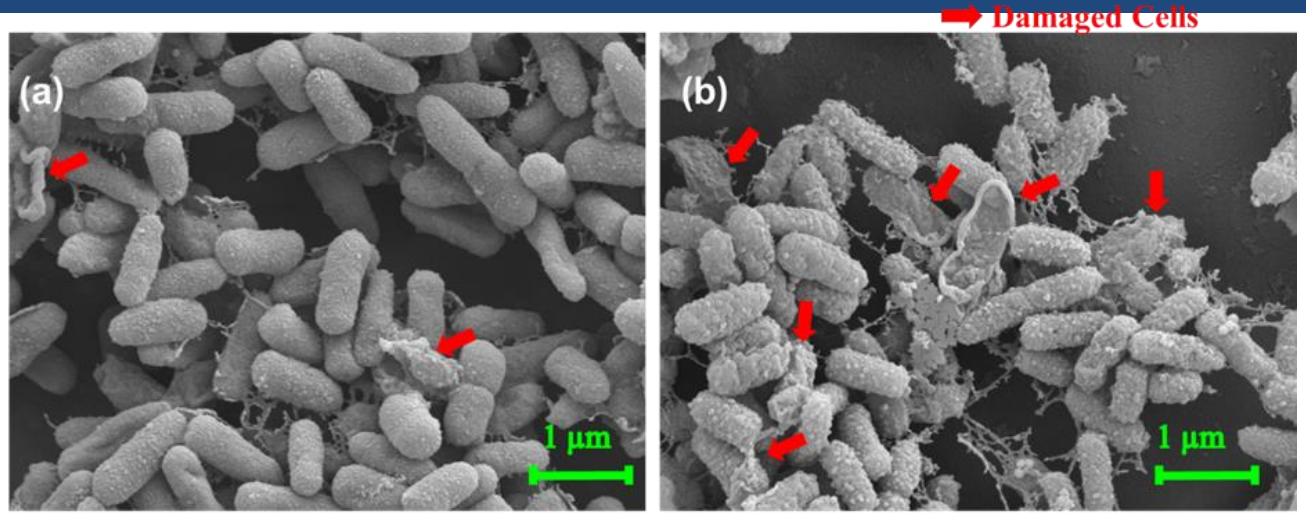
Well Diffusion method





PAW inactivation study on *P. aeruginosa*

Morphology analysis of *P. aeruginosa (Gram Negative)* after PAW treatment

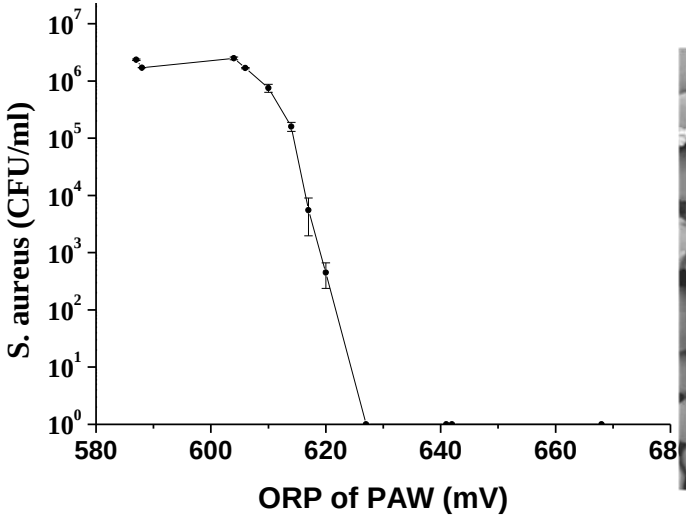


Control PAW Treated

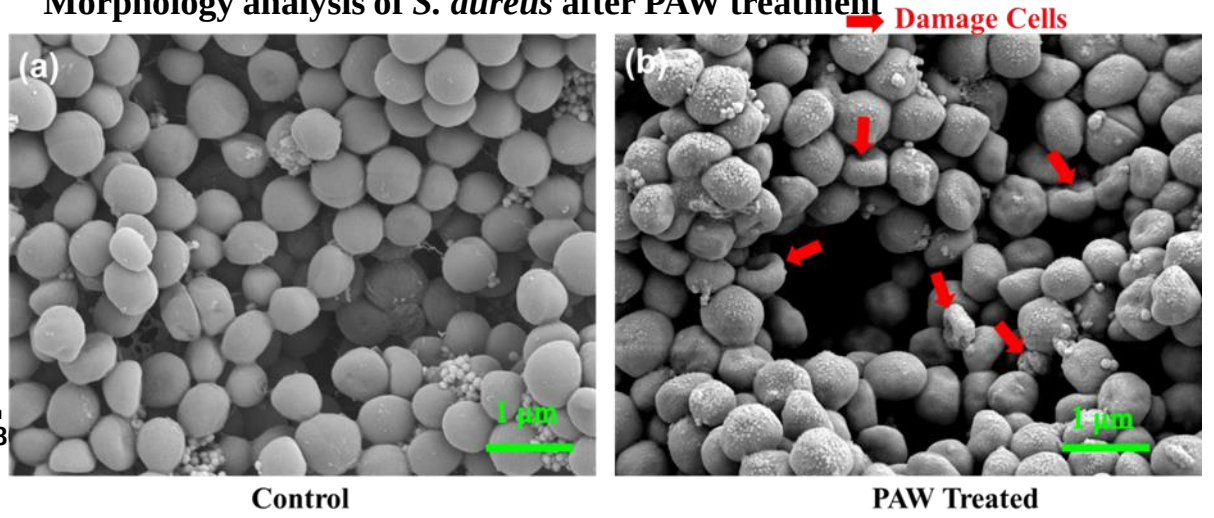
Fluorescence microscopy image of P. aeruginosa after PAW treatment

V. Rathore, D. Patel, Butani, S.K Nema; Investigation of Physicochemical Properties of Plasma Activated Water and its Bactericidal Efficacy. ***Plasma Chem. Plasma Processing* 41,** 871–902 (2021).

PAW- inactivation study on *S. aureus*



Morphology analysis of *S. aureus* after PAW treatment



Fluorescence microscopy image of *S. aureus* after PAW treatment

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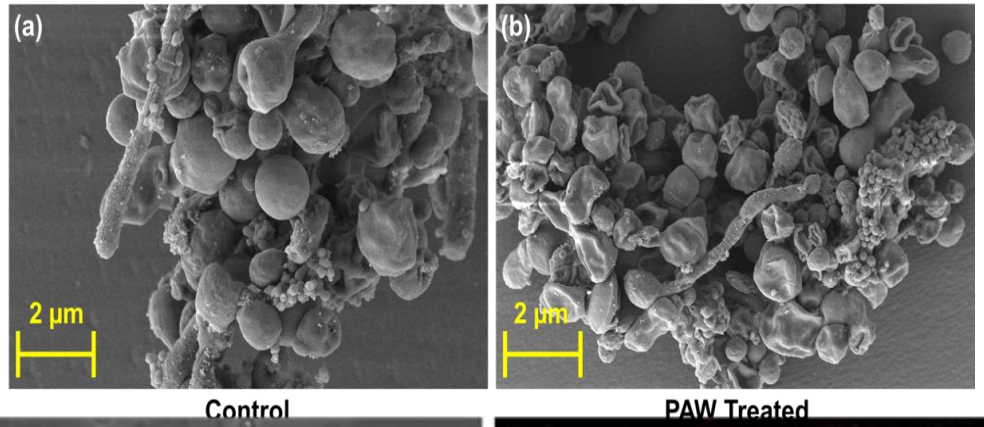
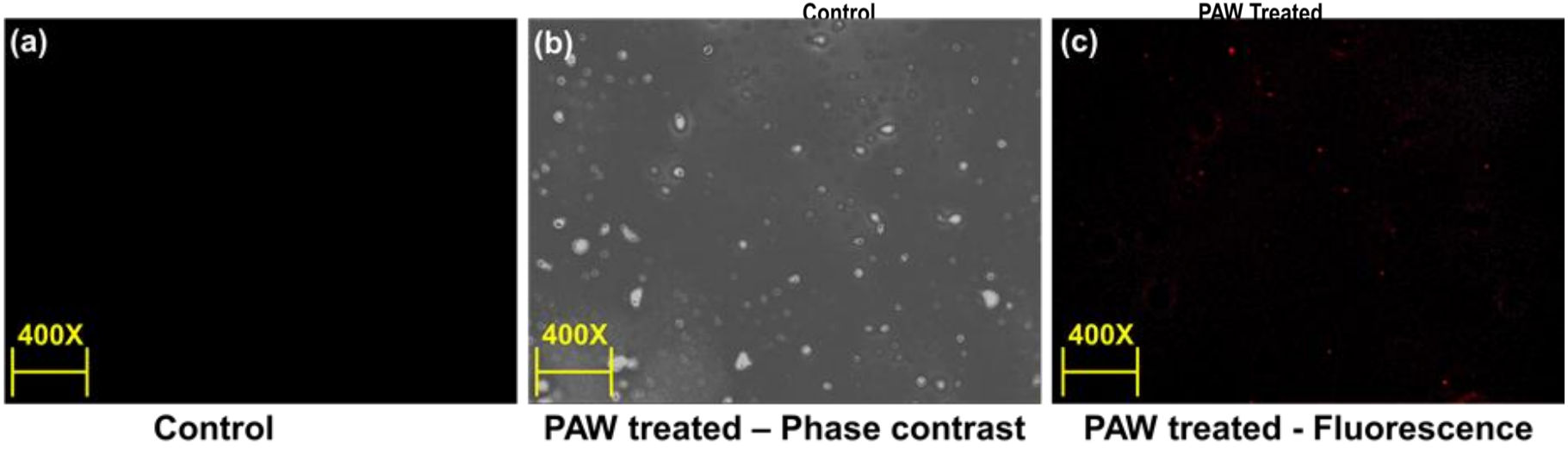
PAW- inactivation study on *C. albicans Fungus cells*

Morphology analysis of *C. albicans Fungus cells* after PAW treatment





Fluorescence microscopy image of *C. albicans* after PAW treatment

V.Rathore, D. Patel, N. Shah, S.K. Nema Inactivation of Candida albicans and Lemon (Citrus limon) Spoilage Fungi Using Plasma Activated Water. ***Plasma Chem. Plasma Processing*** **(2021).**

Conclusion

- APPJ has great potential for biomedical application like cancer treatment, Fungal skin treatment etc .
- More human trials are necessary to establish its clinical uses.
- Multiple plasma jets or micro plasma array can be used for sanitization of surface as well as hand sanitizer.
- Plasma activated medium like water can be used for some of the biomedical applications where direct plasma treatment is not possible.
- Plasma activated water has shown good bactericidal and fungicidal tendency against *P. aeruginosa* and *C. albicans*. Therefore, it has potential to replace presently used disinfectants.

Challenges and Opportunities

Challenges :

- Determine Plasma dose and quantification : Many influencing parameters
- Increasing/ controlling penetration depth of the reactive species
- Ethical permission for using this technology in medical sector
- Challenging to generate cold atmospheric pressure air plasma jet with gas temperature close to or at room temperature.
- Controlling/removing the emission of other extra species like ozone during treatment inside the body

Opportunities :

- Compact and low cost device for treatment like cancer
- Portable device : Blood coagulator during emergency
- Low cost treatment tool for wound healing application in Diabetic foot Ulcers
- Great potential for localized treatment after removal of tumor

Acknowledgment

Team at IPR

- S. Mukherjee
- S. Nema
- Alphonsa Joseph
- Mukesh Ranjan
- Bharathi M
- Ramkrishna Rane
- Akshay Vaid
- Anand Visani
- Rohit Parihar
- Parmesh Maila
- Vikas Rathod

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Thank You

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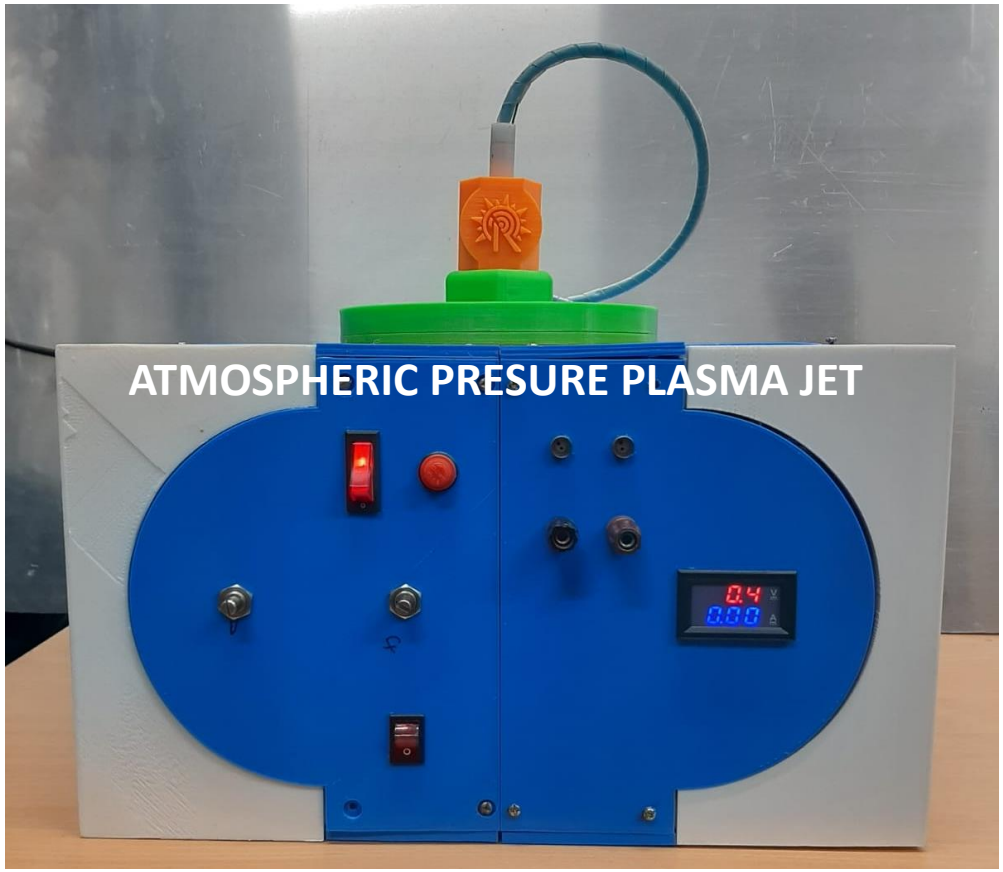

ATMOSPHERIC PRESURE PLASMA JET SET UP DEVELOPED AT IPR