IAEA
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Influential phenotypic traits of living cells & tissues using atm cold plasma source as biocatalysts

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- Mild-Biocatalyst
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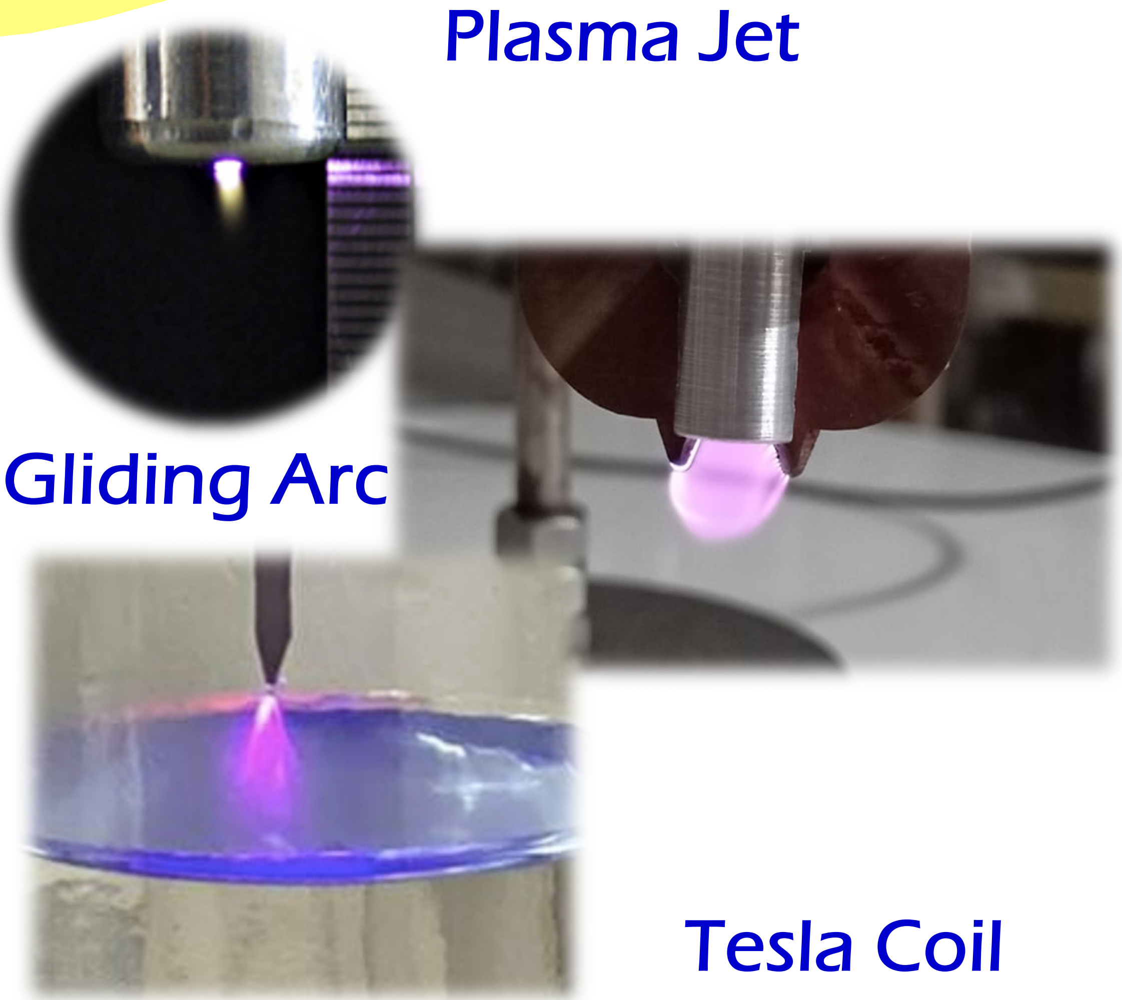
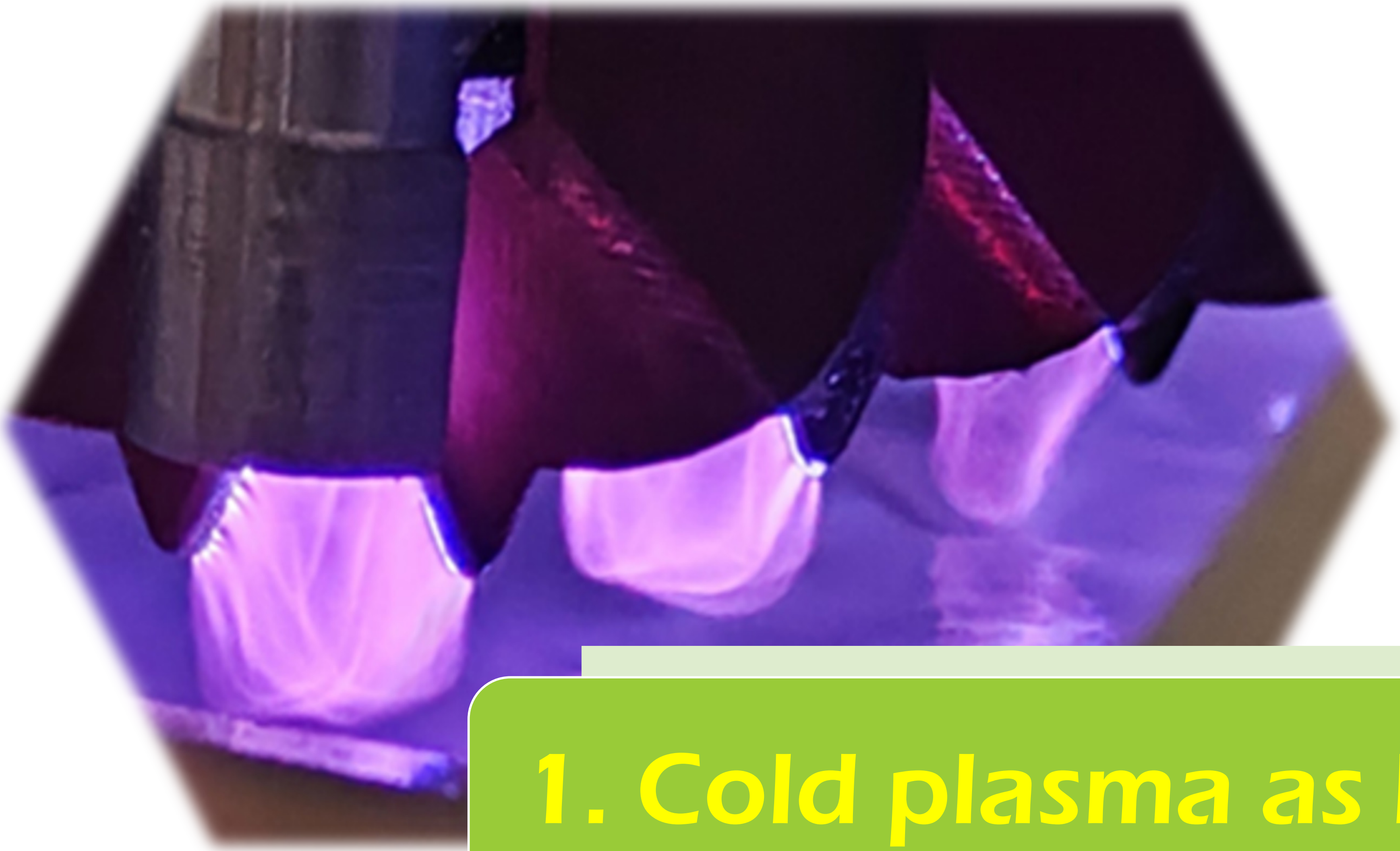
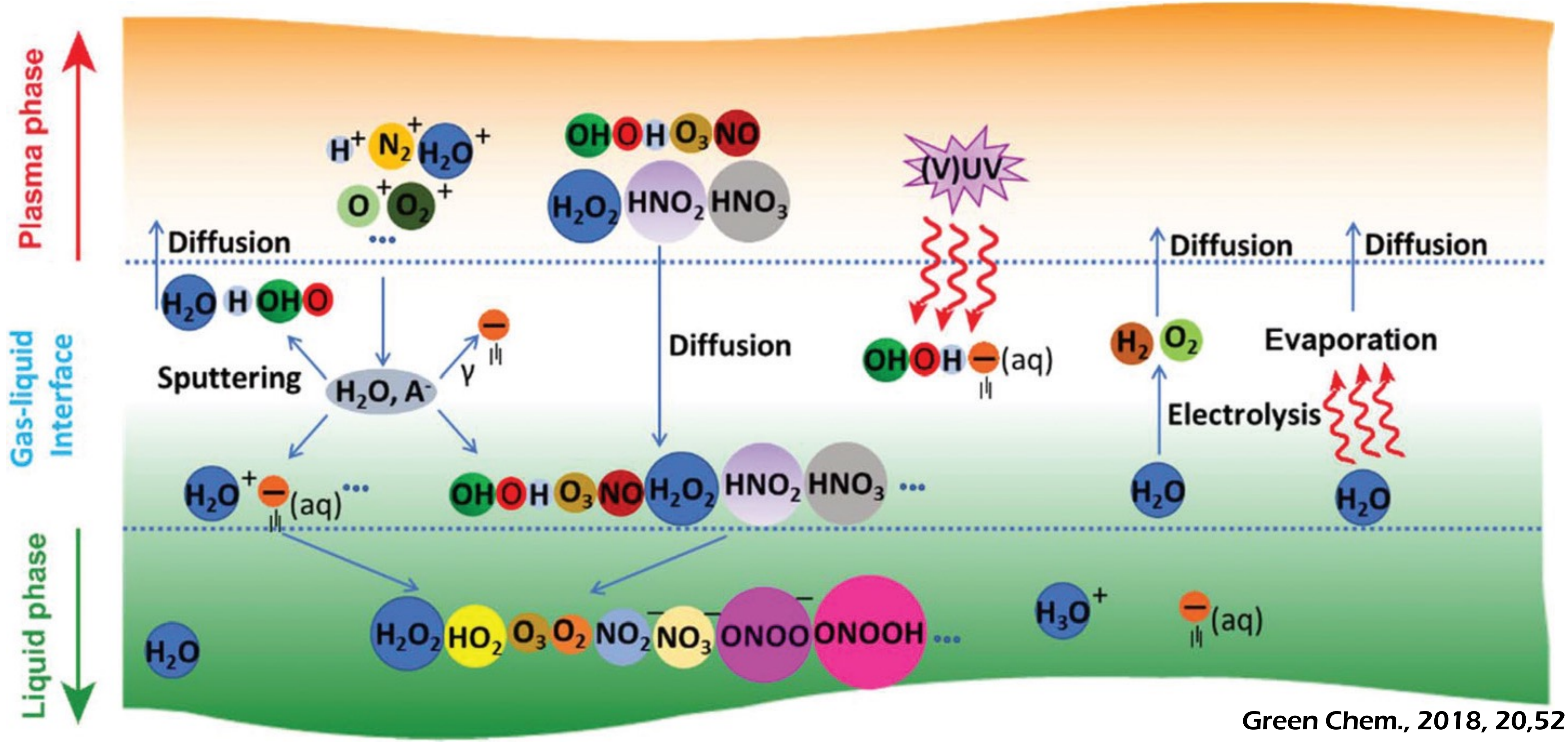




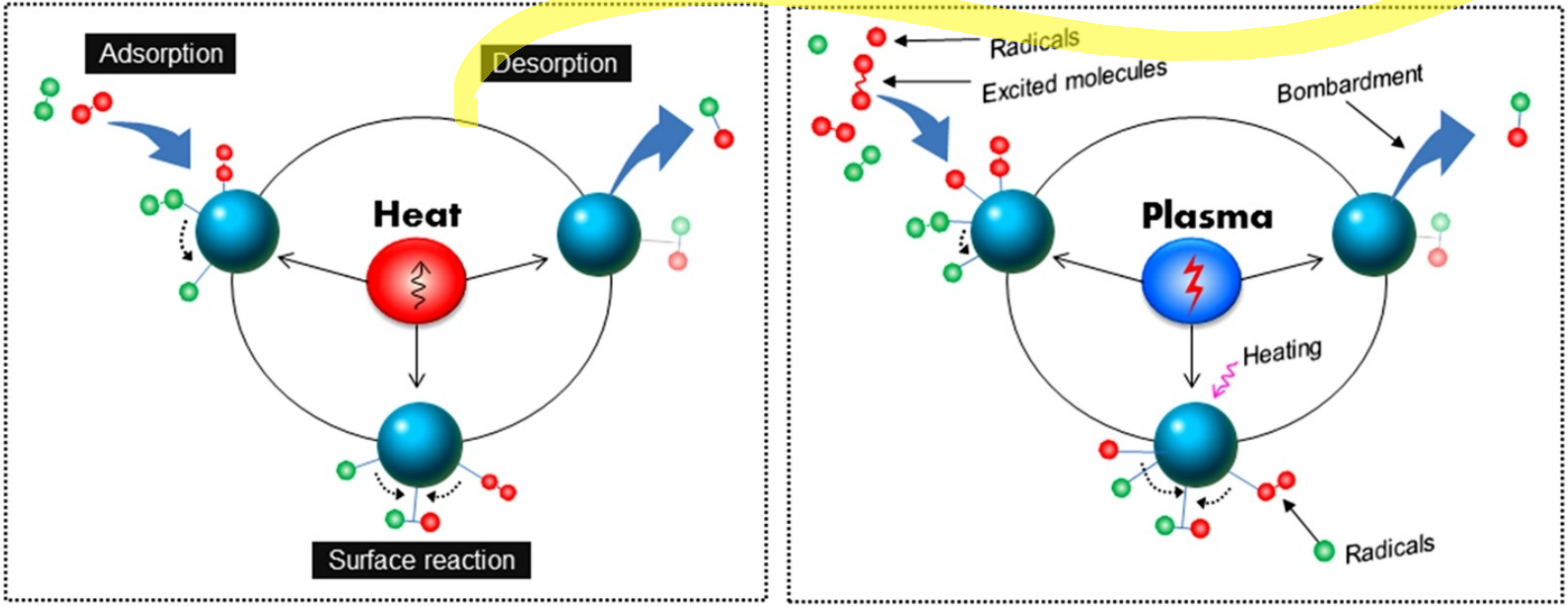
1. Cold plasma as biocatalyst

Mechanisms of delivery of reactive oxygen and nitrogen species (RONS)



Green Chem., 2018, 20,5276

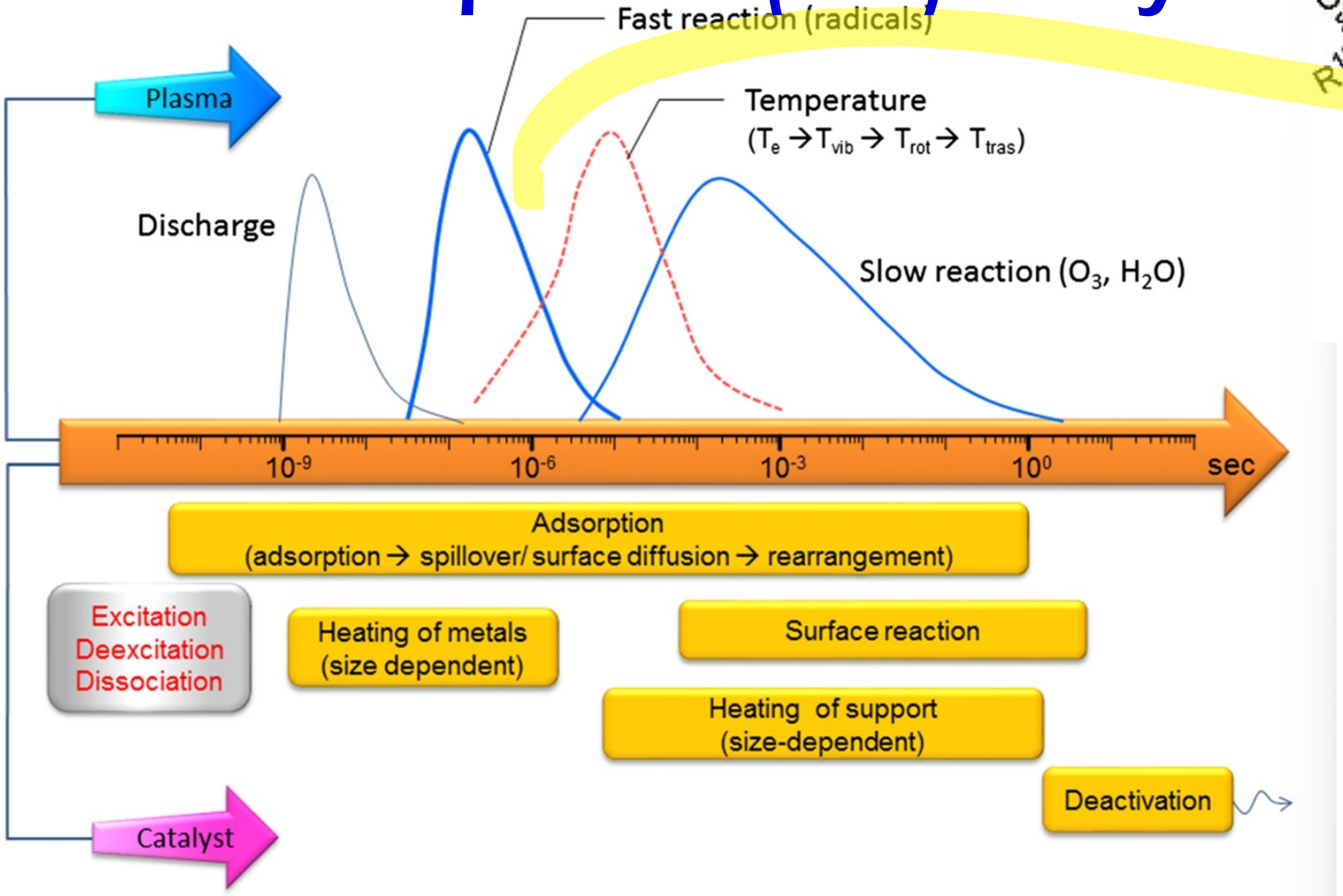
(Bio)catalysis



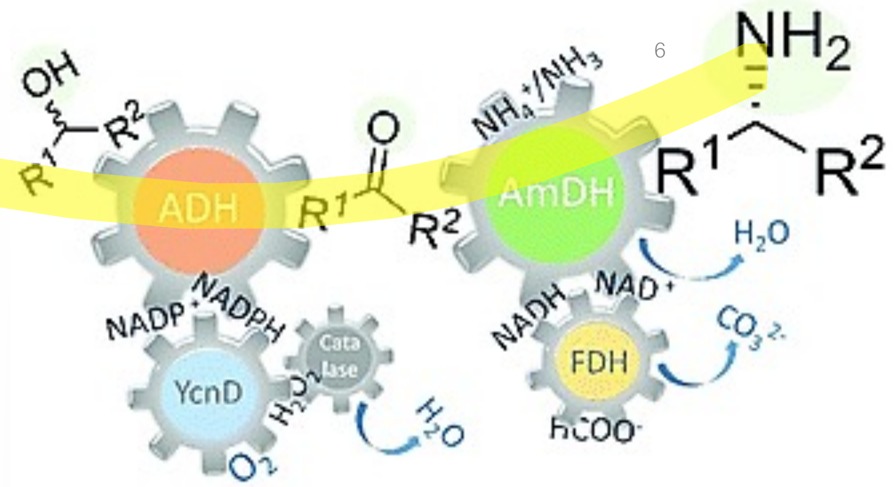
(a) Thermal catalysis (b) Plasma-catalysis

Kim H., et al Plasma Chem Plasma Process, 2015

Thus cold plasma (Bio)catalysis

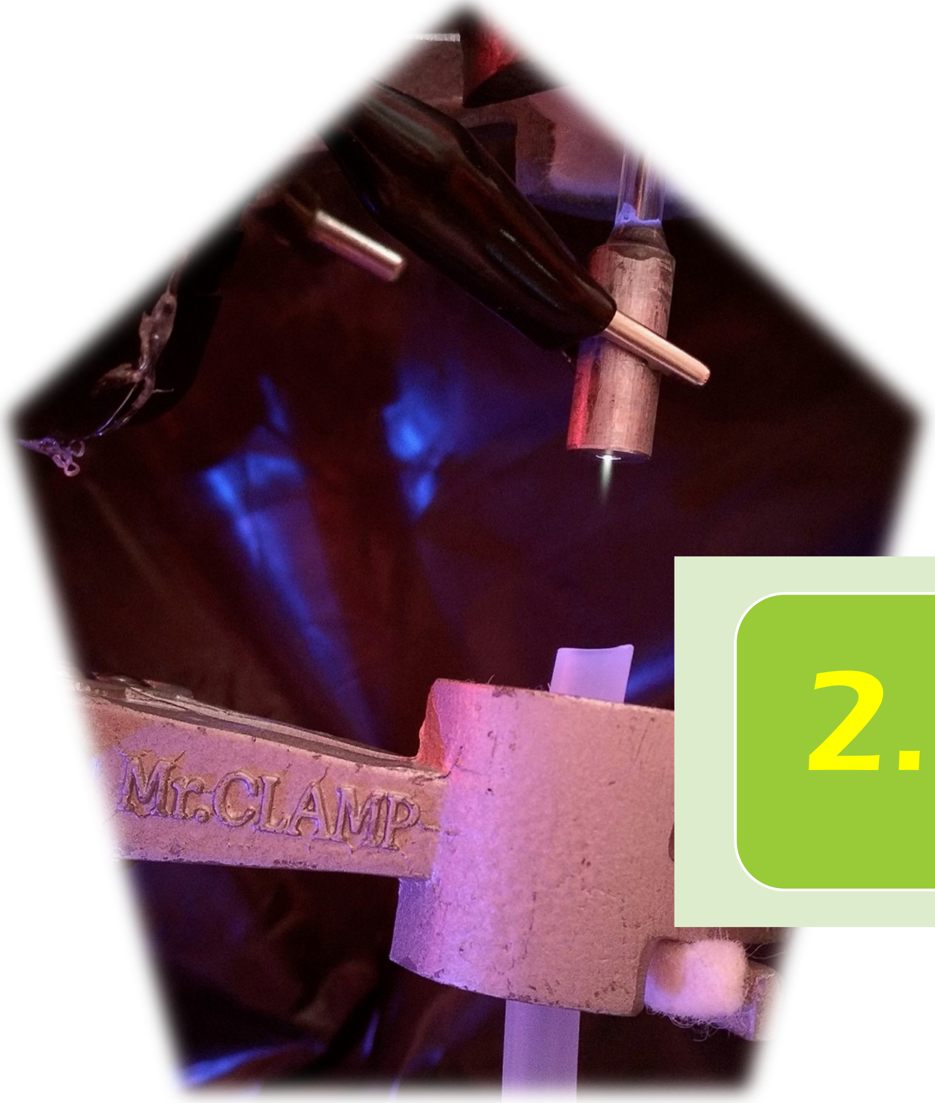


Kim H., et al Plasma Chem Plasma Process, 2015



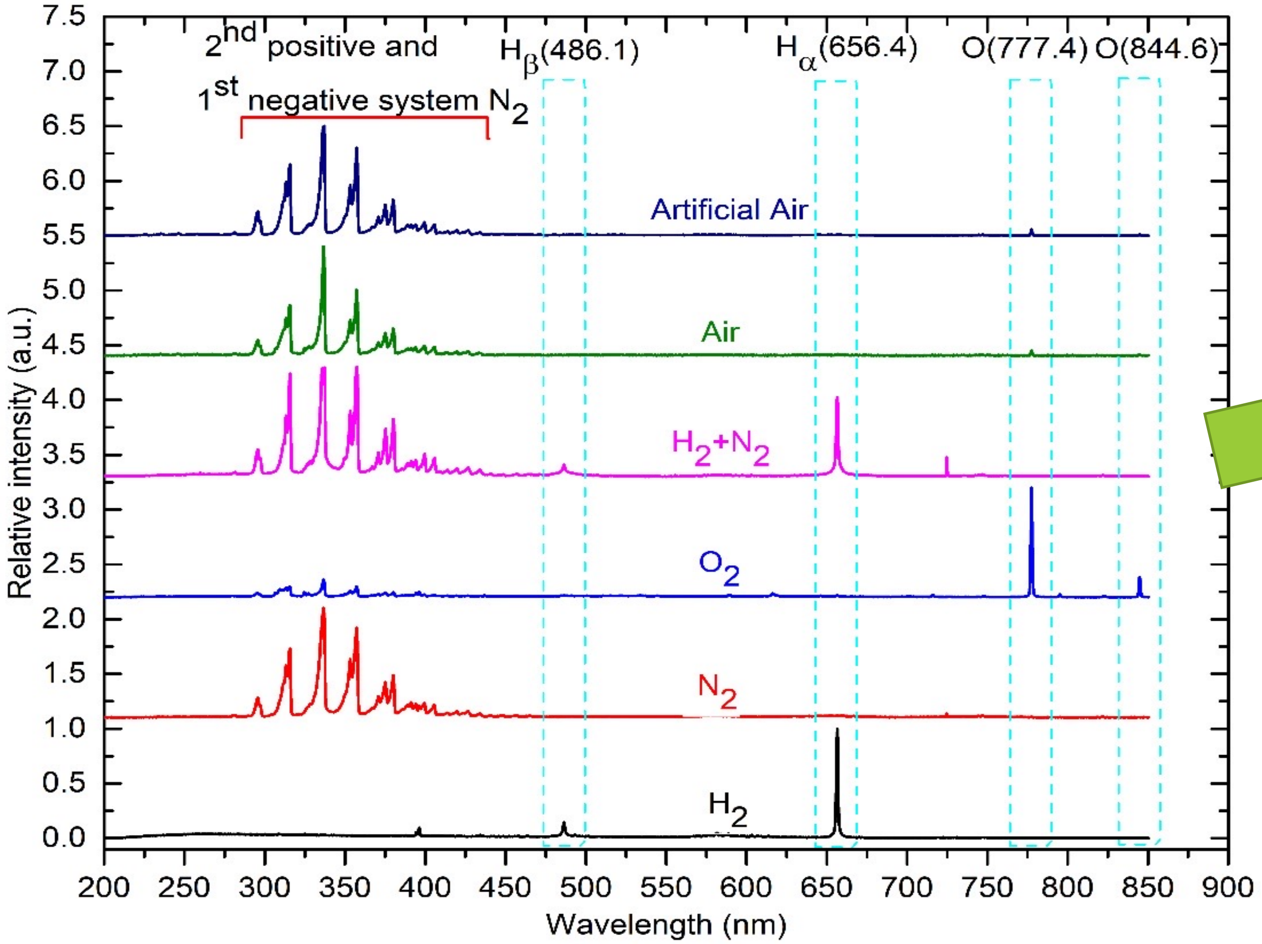
Tanya K., et al Org. Biomol. Chem., 2017

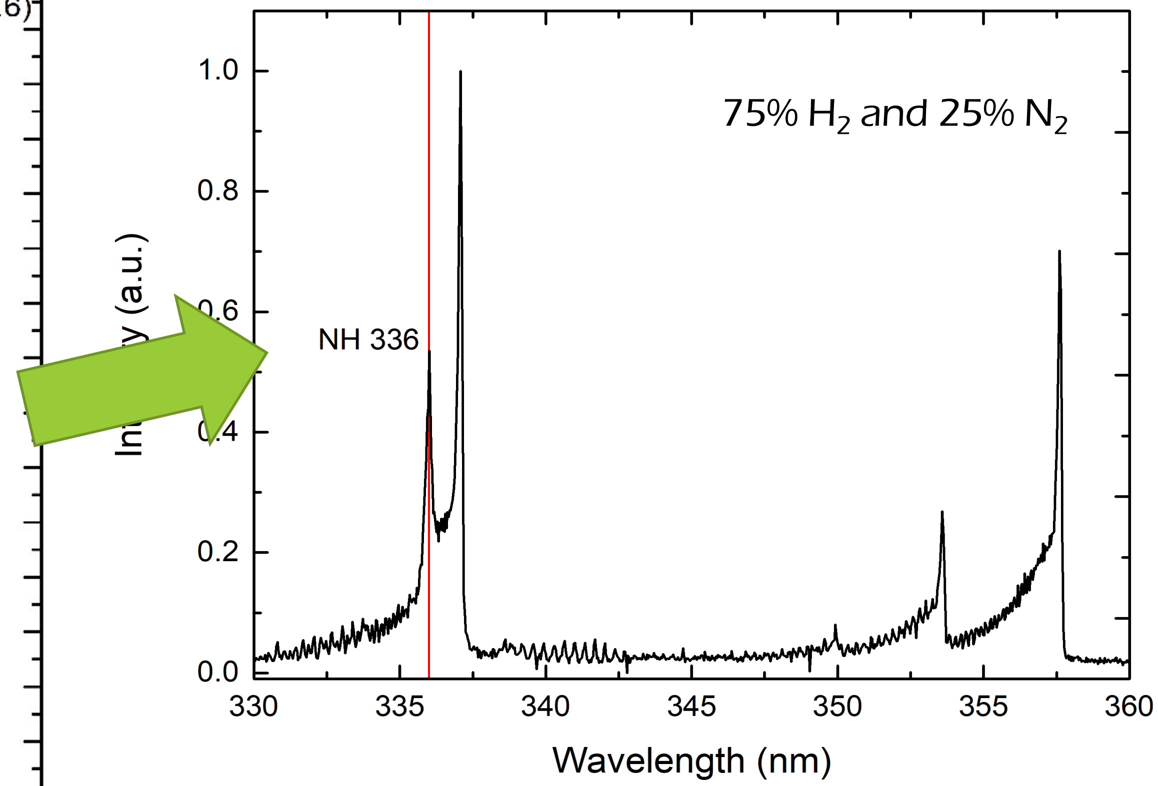
- Radicals generation
- Optimized condition
- Equivalent dose
- Upscaling/Invention/ Application



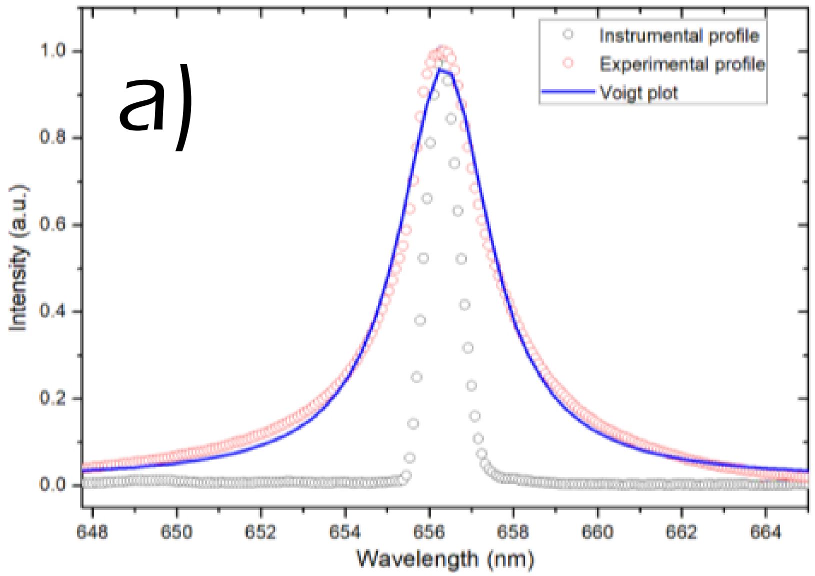
2. Mild Biocatalyst

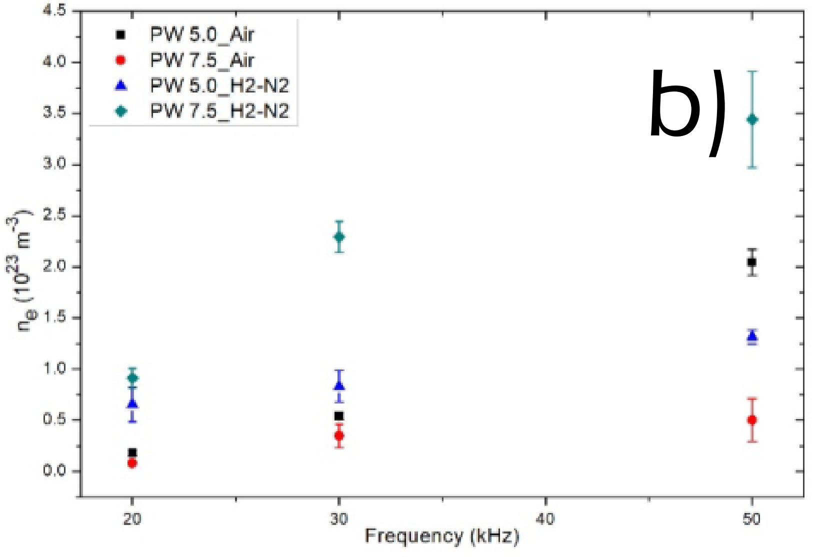
Plasma generation & analysis





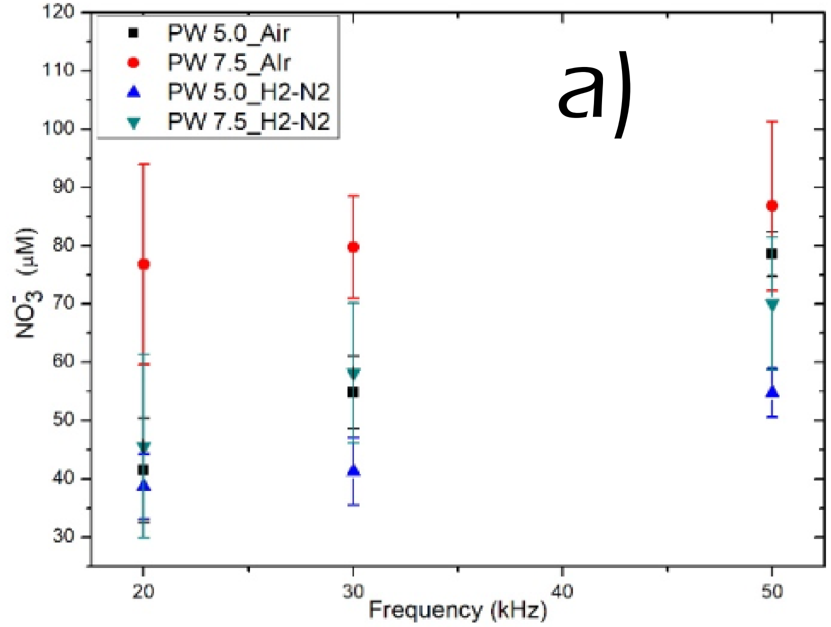
(a) H_α fit and (b) n_e as a function of pulse width & pulse repetition rate.

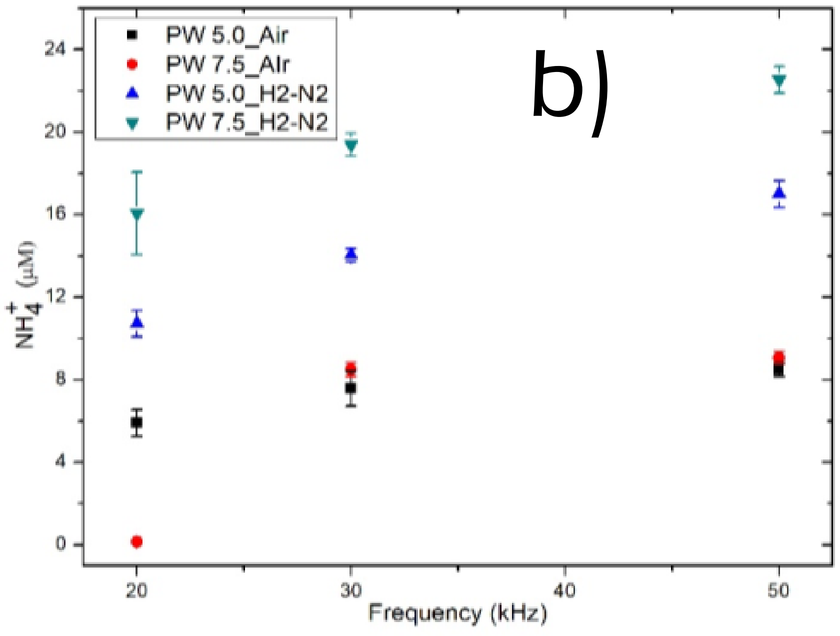




$$n_e[m^{-3}] = 10^{23}\left(\frac{w_s[nm]}{1.098}\right)^{1.471}$$

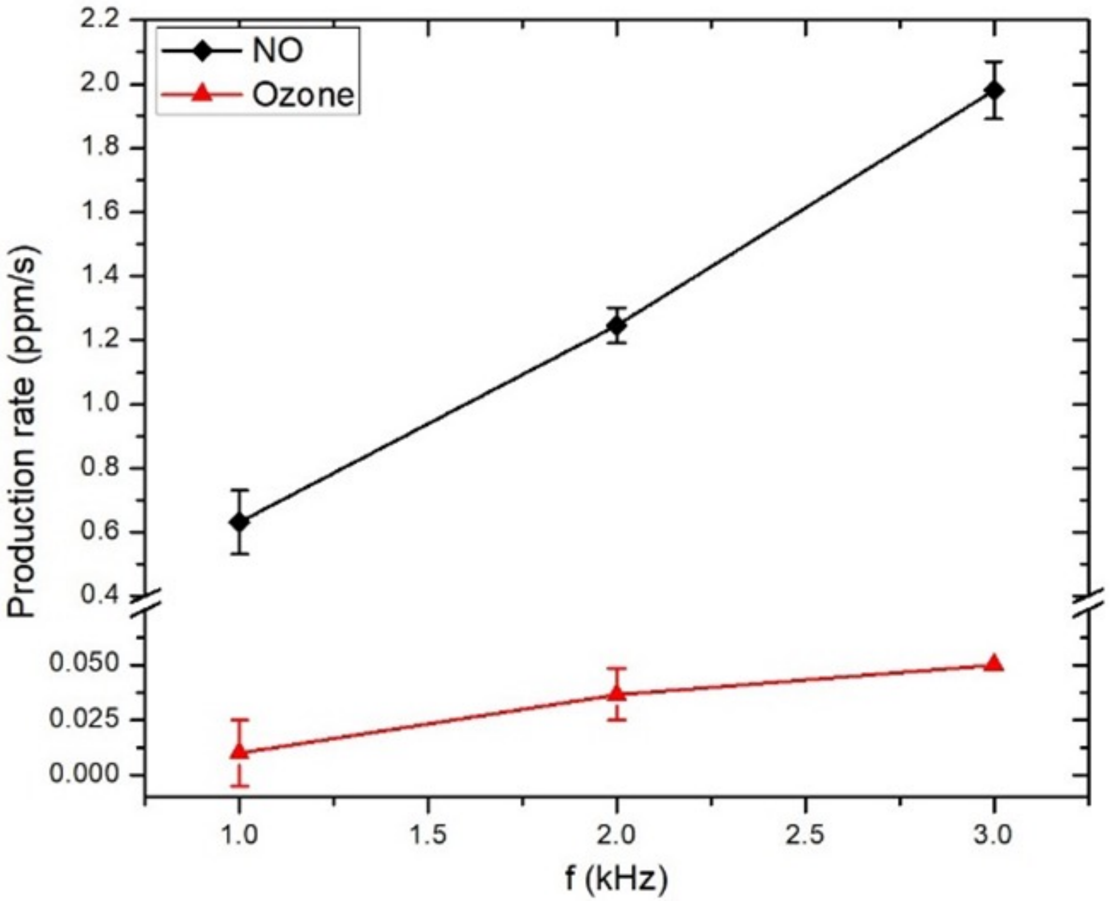
(a) nitrate and (b) ammonium conc. in media as a function of pulse repetition rate.





Afterglow (air) Plasma chemistry

$O_2(a)+O_3 \rightarrow O_2+O_2+O$	$(1.00\times10^{-10})\ \exp(-0/T_g)\ [cm^3/s]$
$N+OH \rightarrow H+NO$	$(4.70\times10^{-11})\ \exp(-0/T_g)\ [cm^3/s]$
$N+O_2 \rightarrow NO+O_2$	$(8.20\times10^{-11})\ \exp(-410/T_g)\ [cm^3/s]$
$OH+O \rightarrow H+O_2$	$(1.81\times10^{-11})\ ((T_g/300)^{-0.31})\ \exp\ (177/T_g\ [cm^3/s]$
$H+O_3 \rightarrow OH+O_2$	$(2.71\times10^{-11})\ ((T_g/300)^{0.75})\ \exp(-0/T_g)\ [cm^3/s]$
$OH+O \rightarrow H+O_2$	$(1.81\times10^{-11})\ ((T_g/300)^{-0.31})\ \exp(177/T_g)\ [cm^3/s]$
$H+NO_2 \rightarrow OH+NO$	$(4.00\times10^{-10})\ \exp(-340/T_g)\ [cm^3/s]$
$O_2+N \rightarrow NO+O$	$(3.30\times10^{-12})\ (T_g/300)\ \exp(-3150/T_g)\ [cm^3/s]$
$N+NO_2 \rightarrow NO+NO$	$(1.33\times10^{-12})\ \exp(220/T_g)\ [cm^3/s]$
$NO+N \rightarrow N_2+O$	$(8.20\times10^{-11})\ \exp(-410/T_g)\ [cm^3/s]$
$NO+O_3 \rightarrow NO_2+O_2$	$(4.30\times10^{-12})\ \exp(-1560/T_g)\ [cm^3/s]$
$NO+O+N_2 \rightarrow NO_2+N_2$	$(1.00\times10^{-31})\ ((T_g/300)^{-1.6})\ [cm^6/s]$
$NO+NO_3 \rightarrow NO_2+NO_2$	$(1.80\times10^{-11})\ ((T_g/300)^{-0.31})\ \exp(177/T_g)\ [cm^3/s]$



P. Thana et al. Heliyon 2019

Medium chemistry

NO and O_3 dissolve

$$O_3 + H_2O \rightarrow H_2O_2 + O_2$$

$$H\bullet + O_2 \rightarrow HO_2\bullet$$

$$HO_2\bullet \leftrightarrow H^+ + O_2\bullet^- \quad \text{(pKa = 4.8)}$$

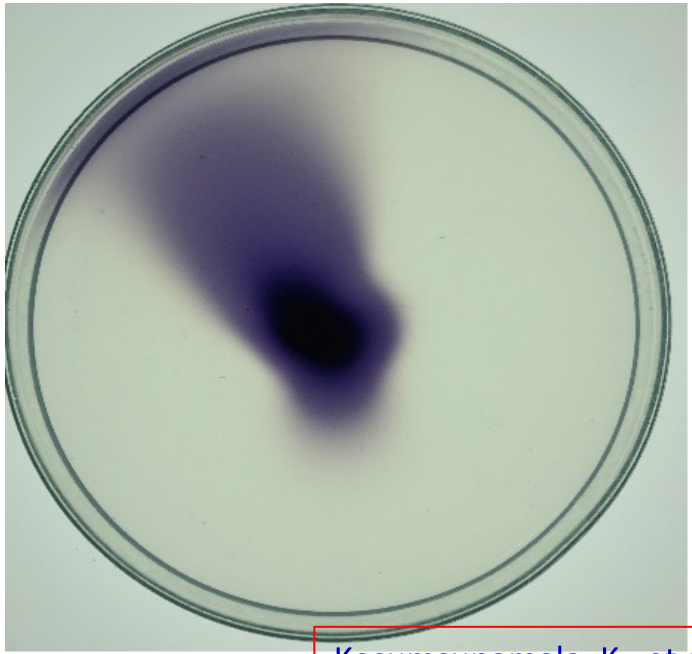
$$4NO + O_2 + 2H_2O \rightarrow 4H^+ + 4NO_2^-$$

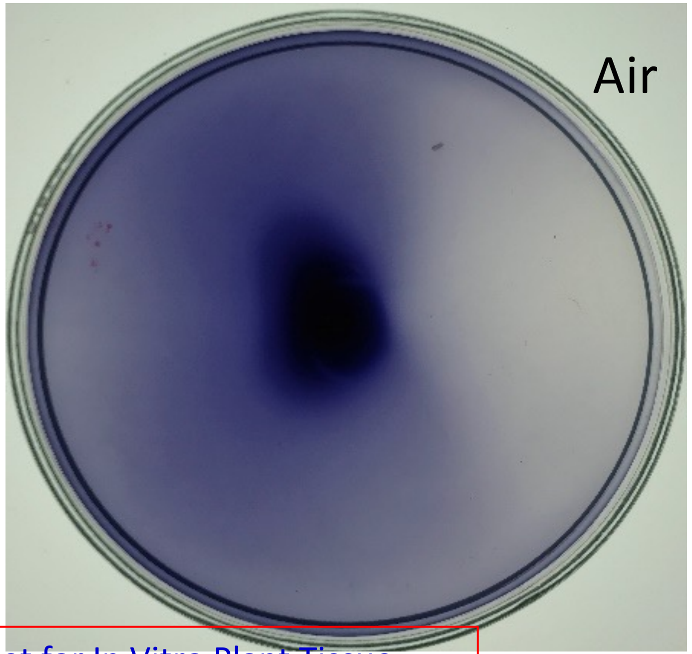
$$2NO_2 + H_2O \rightarrow 2H^+ + NO_2^- + NO_3^-$$

reduced pH

Substance	Formula	Oxidation State of Nitrogen
Ammonia	NH_3	-3
Hydrazine	N_2H_4	-2
Hydride	N_2H_2	-1
Dinitrogen gas	N_2	0
Nitrous oxide	N_2O	+1
Nitric oxide	NO	+2
Dinitrogen trioxide	N_2O_3	+3
Nitrogen dioxide	NO_2	+4
Nitrogen Pentoxide	N_2O_5	+5

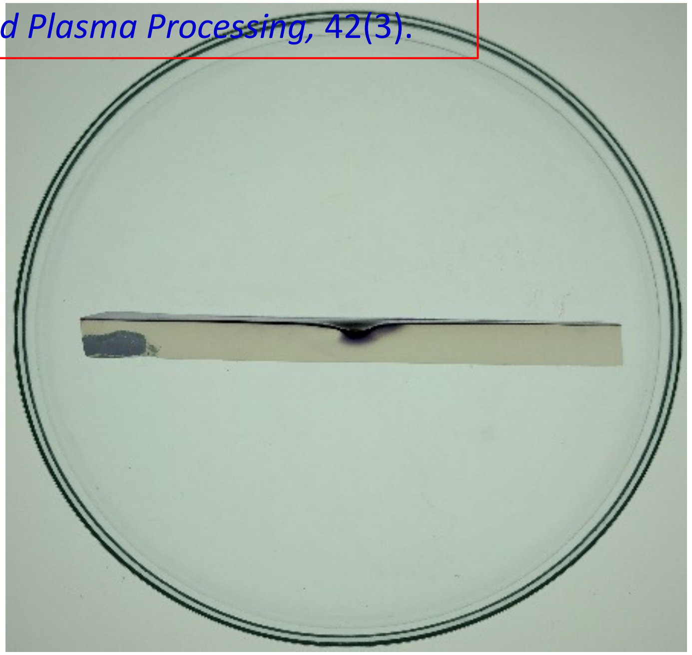
$75\%\ H_2 + 25\%\ N_2$





Top view,

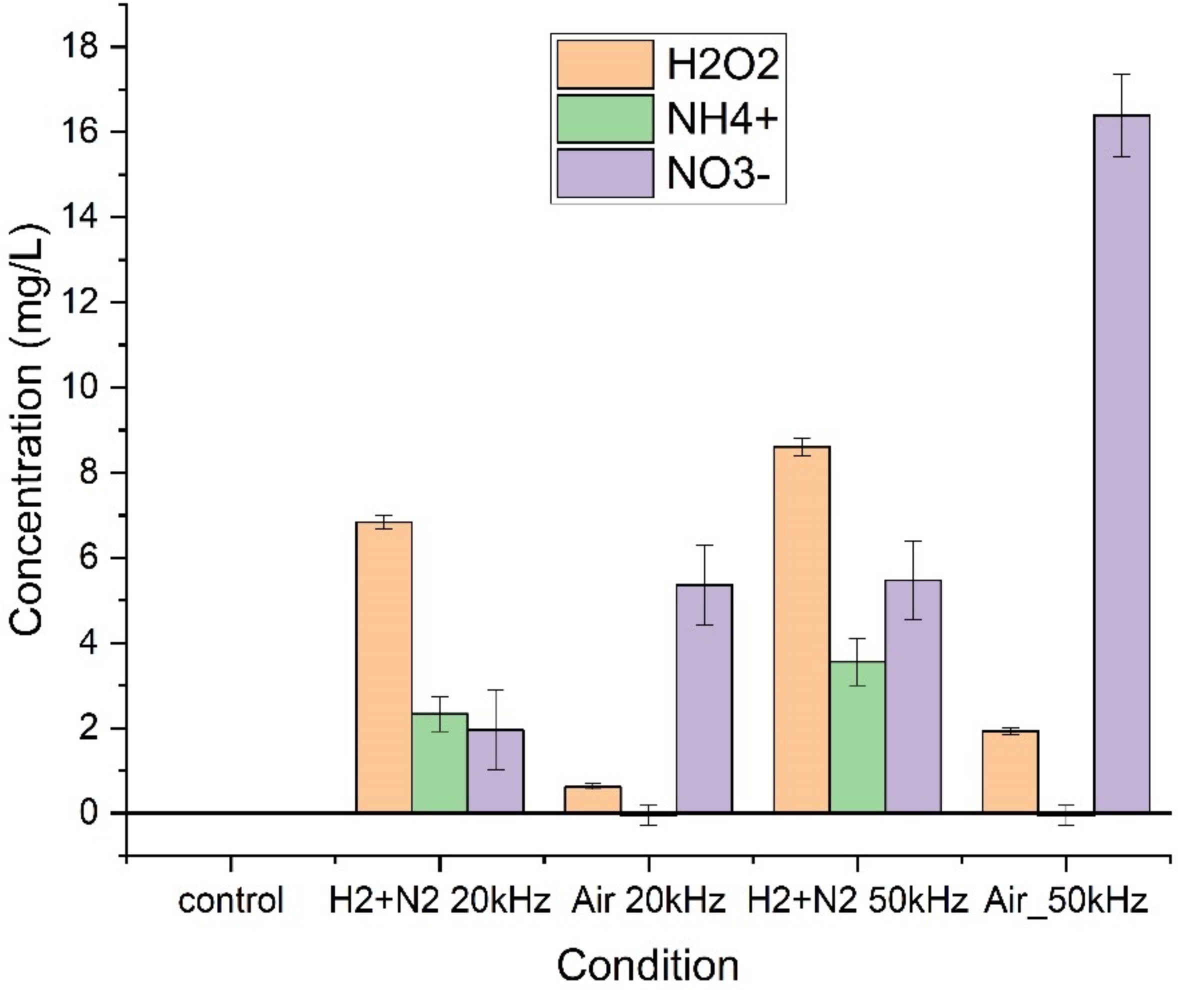
Kosumsupamala, K., et al. (2022) Air to H2 N2 Pulse Plasma Jet for In Vitro Plant Tissue Culture Process: Source Characteristics *Plasma Chemistry and Plasma Processing,* 42(3).



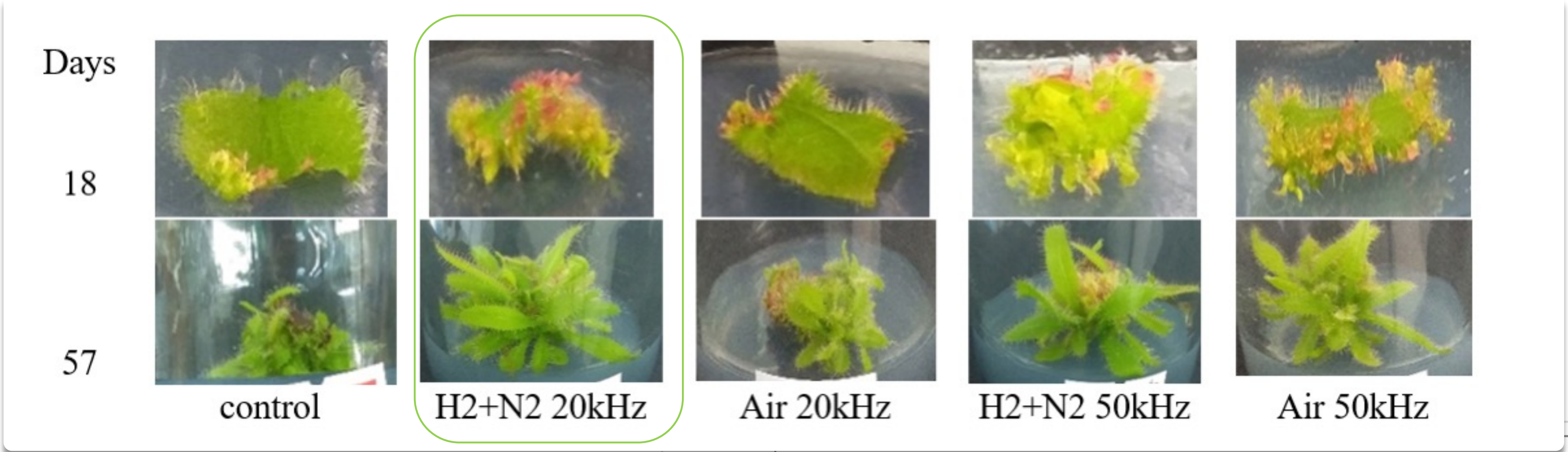
X-section

Gap = 5 mm,
Flow = 4 slm
0.5% Gel
Treatment = 1 min

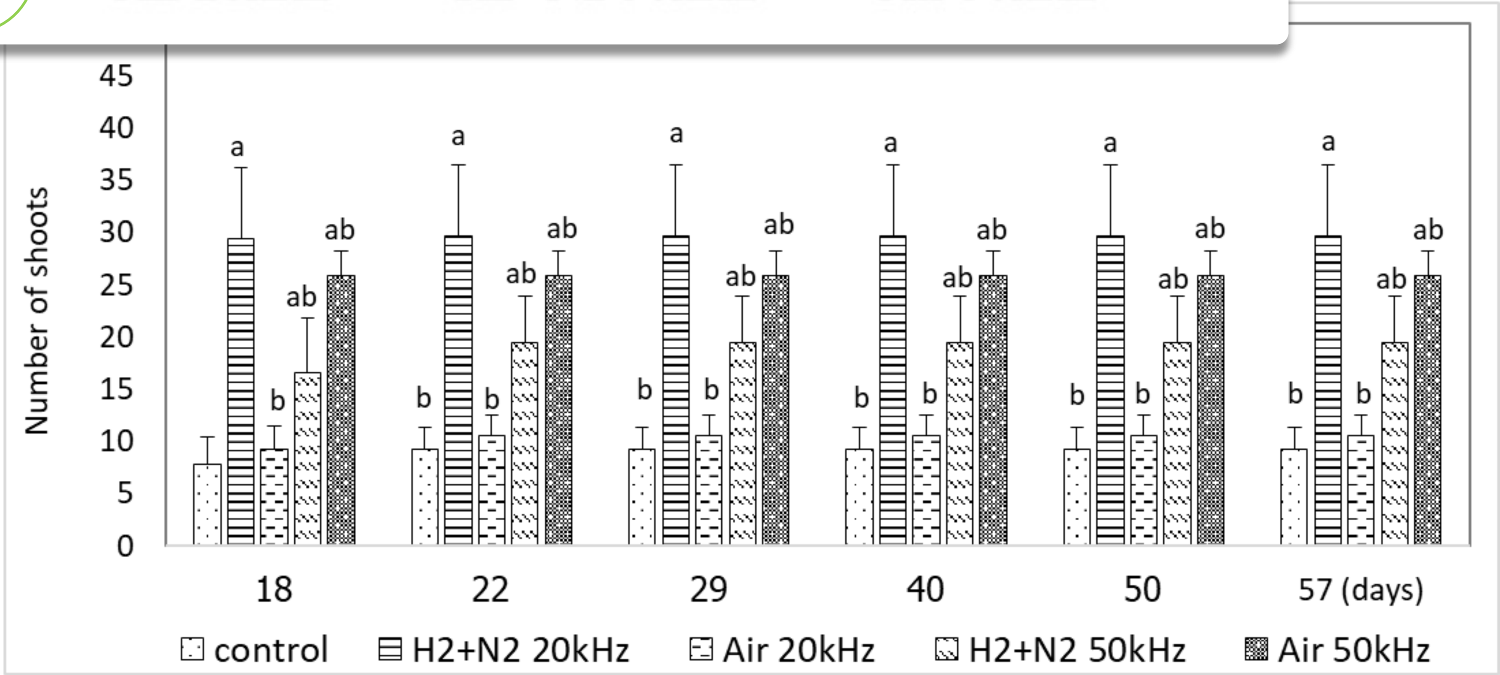
Plasma (radicals) in-vitro



Drosera Adelae leaf explant

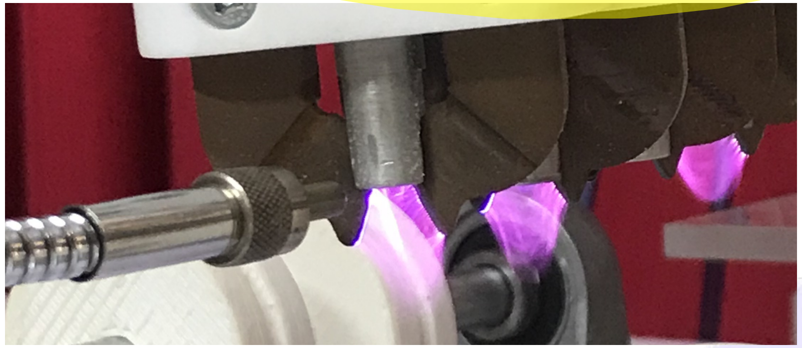


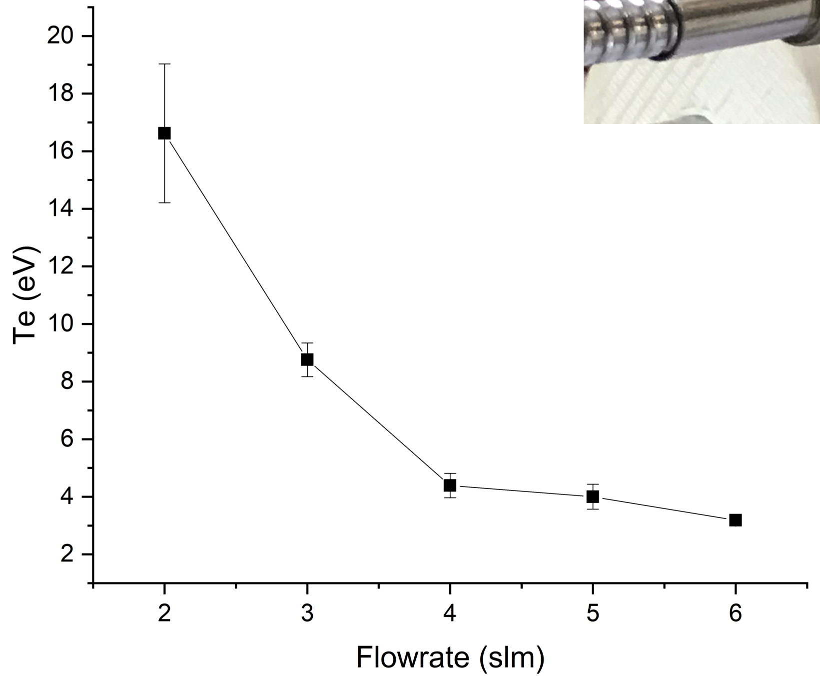
- Leaf explant of Drosera adelae cut into pieces; 1.0×1.5 cm.
- Cultured in the medium treated with and without plasma with 5 replications.
- Incubated under a 16-h photoperiod at 3,000 lux (Daylight LED tubes T8; 14 W) and 25˚C.



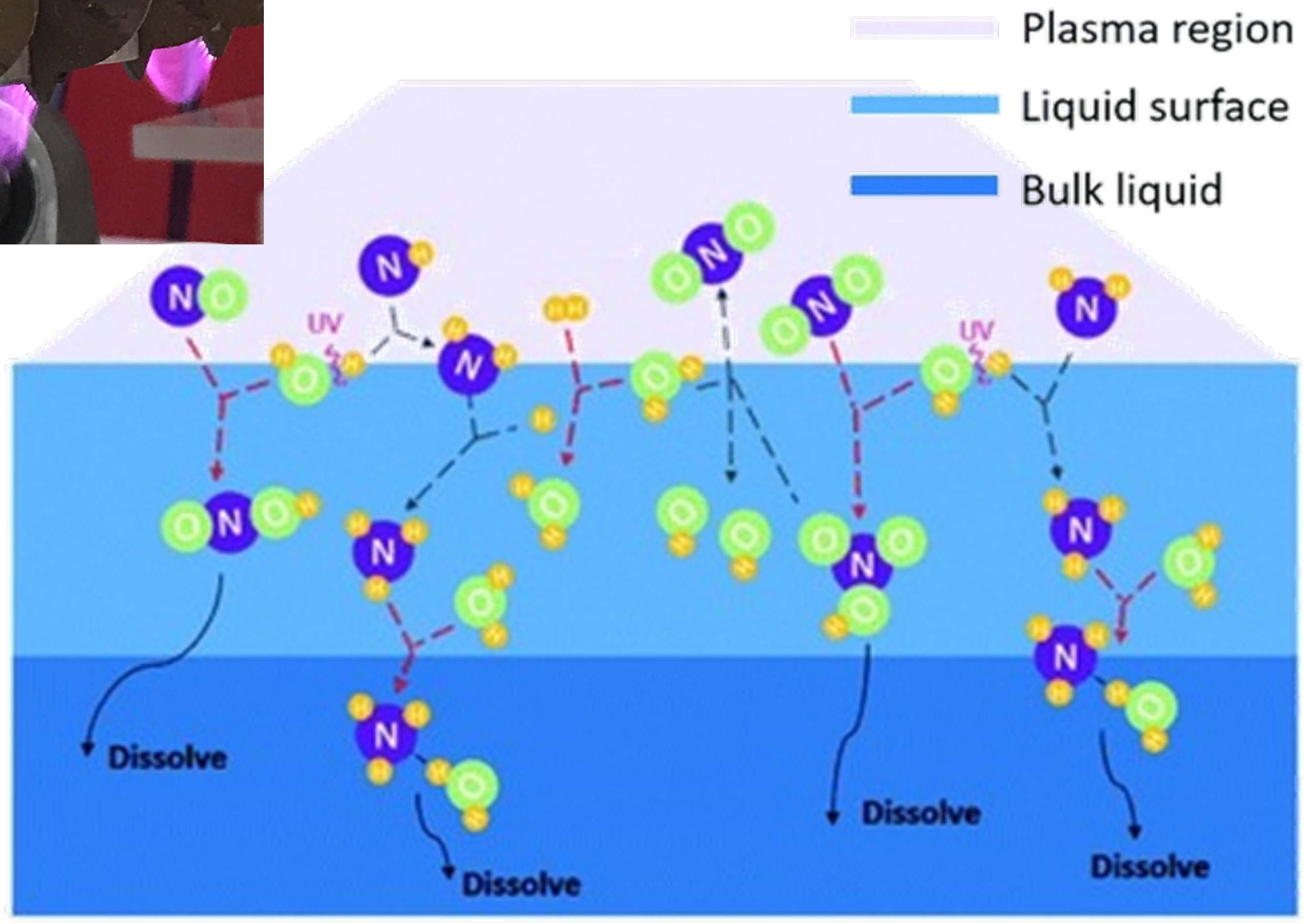
Gliding Arc Plasma

Translational (Hot Non-thermal) Plasma

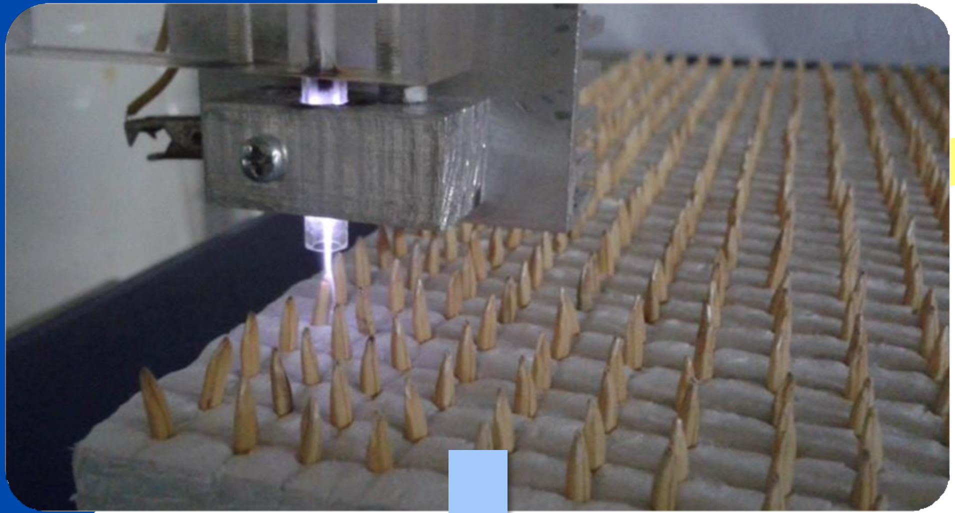


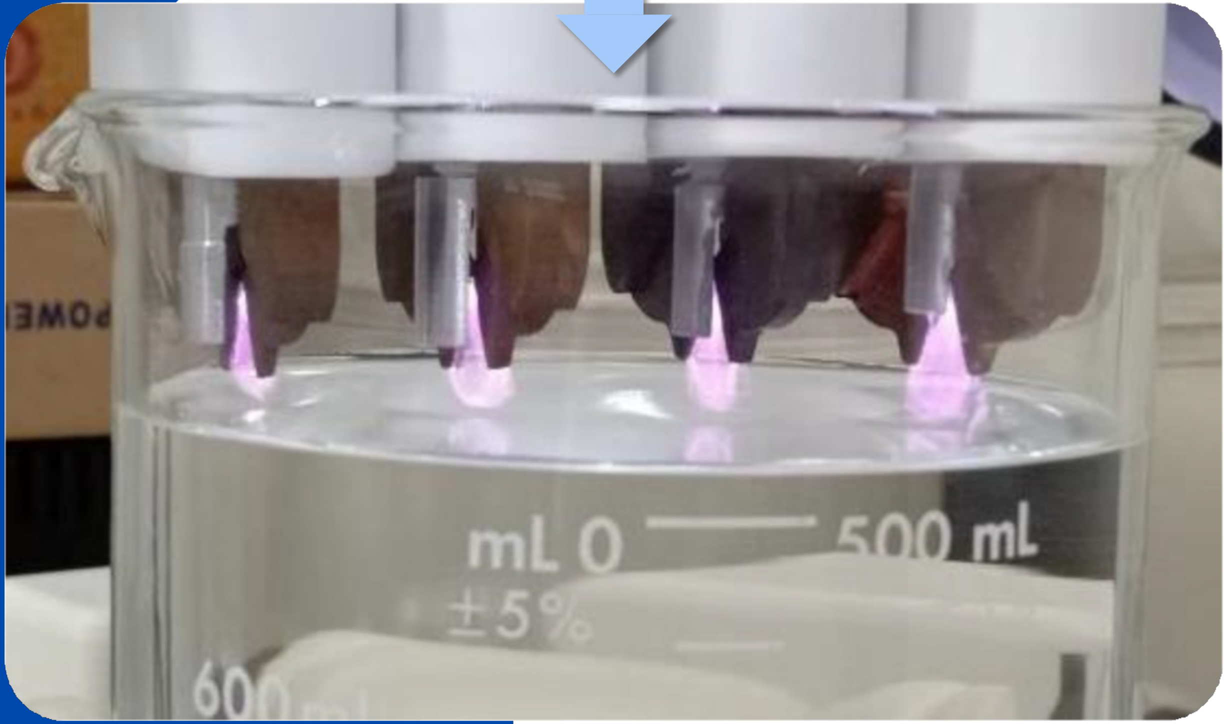


Spectrometer: Exemplar LS; BWTEK Inc., United States (200 to 850 nm) with Integration time: 5000 ms



Typically plants need;





Ideal conditions

for plant germination and growth:
pH : 4.5 – 6.5
NO_2^-/NO_3^- : 50 - 300 ppm
H_2O_2 : 0.1 – 10 ppm

Plasma treatment

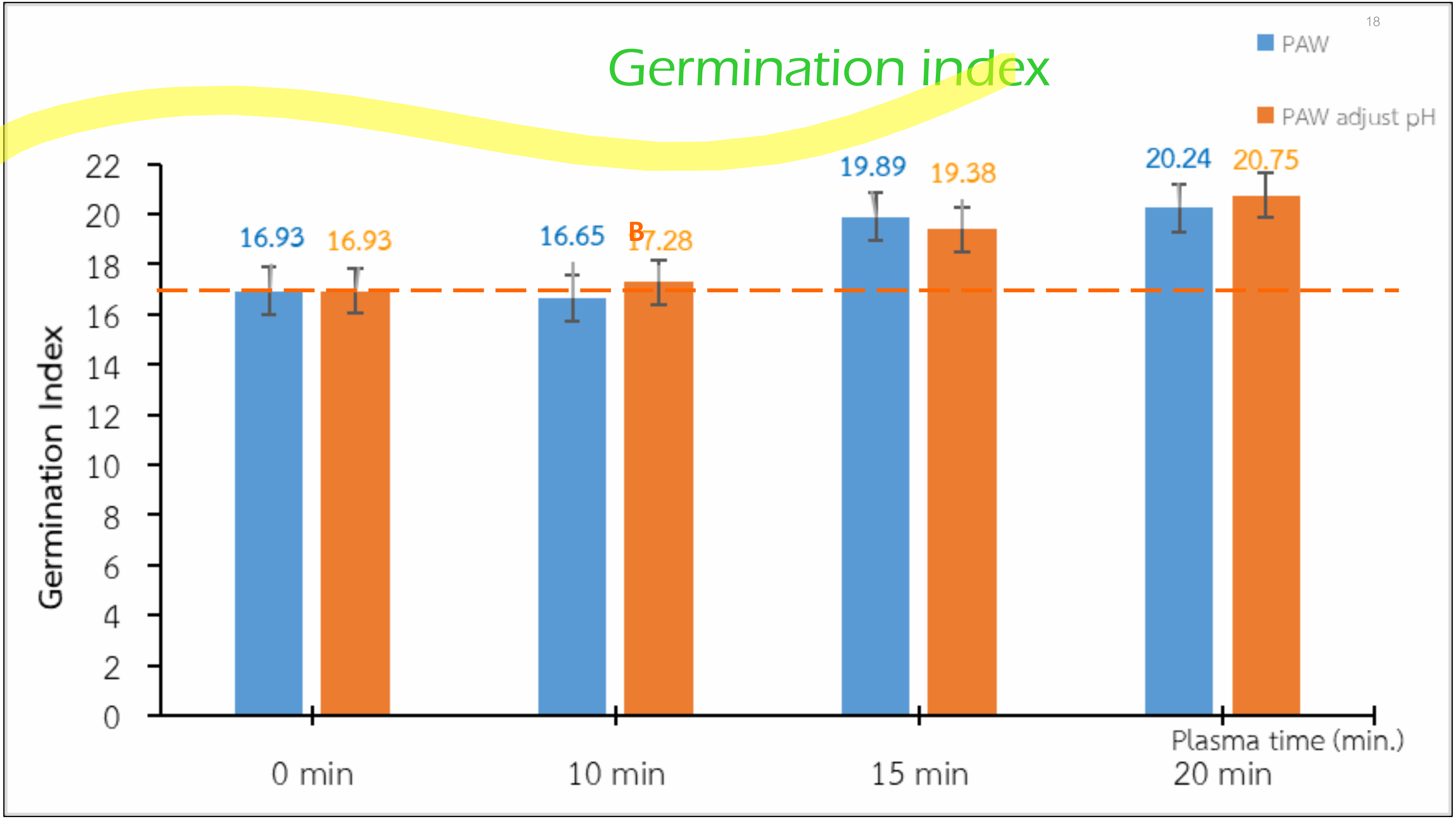
of water stimulates growth making:
pH: 2.5 – 6.5
NO_2^-/NO_3^- : 10 - 500 ppm
H_2O_2 : 0.01 - 100 ppm

Source: Fridman G., IFFM 2015

Radicle Emergence

Time	Control	10 min	15 min	20 min
24 hr				
48 hr				
72 hr				

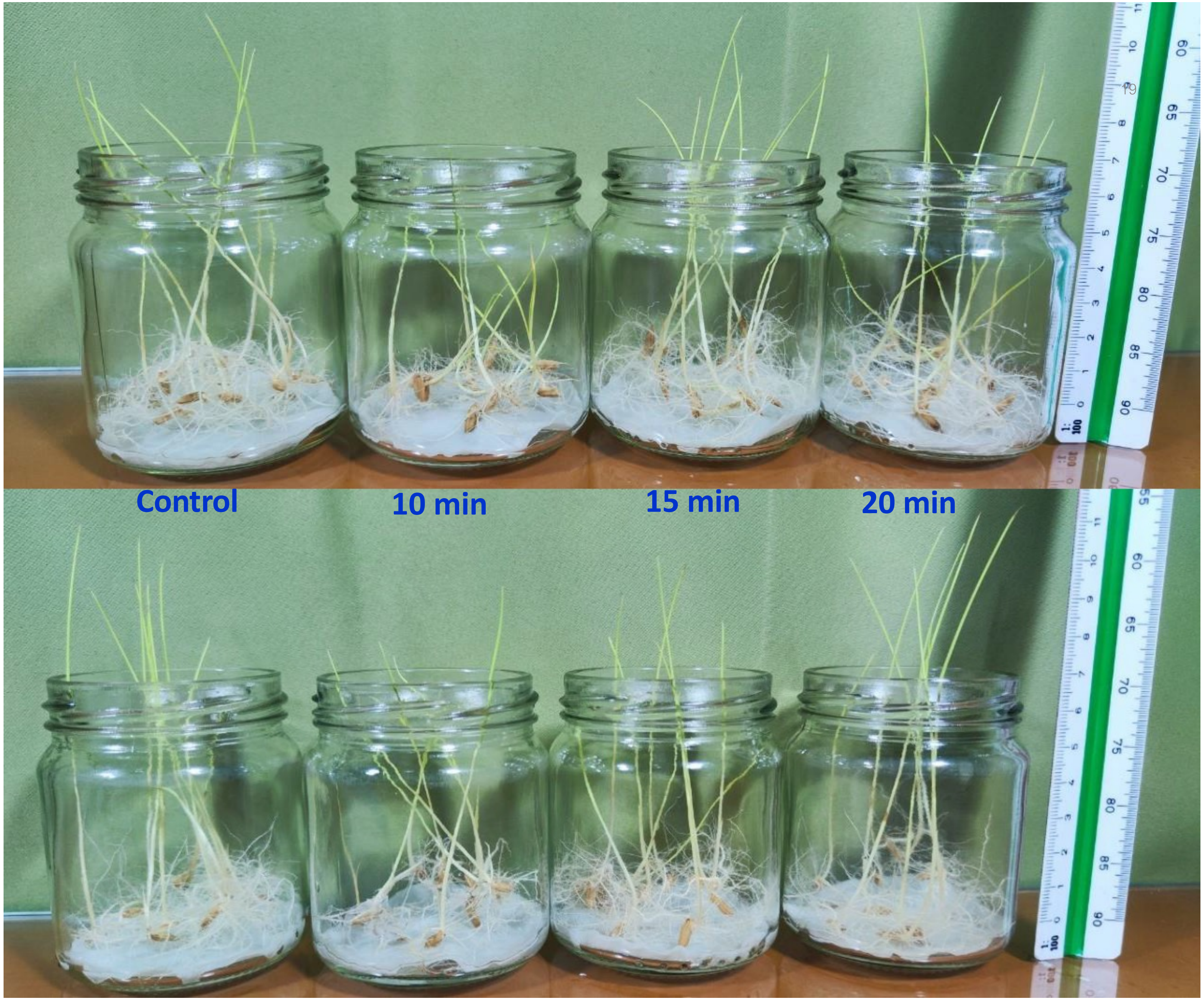
Germination index



Rice germination at 10 day

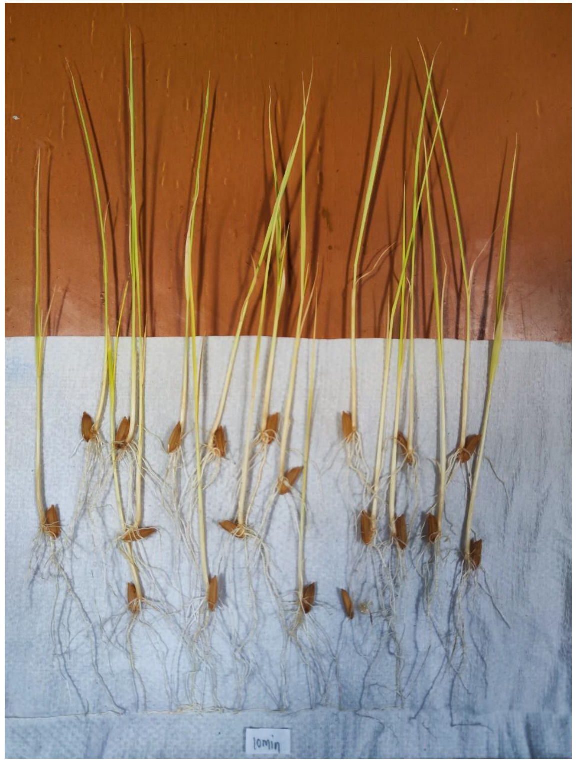
PAW

PAW adjust pH



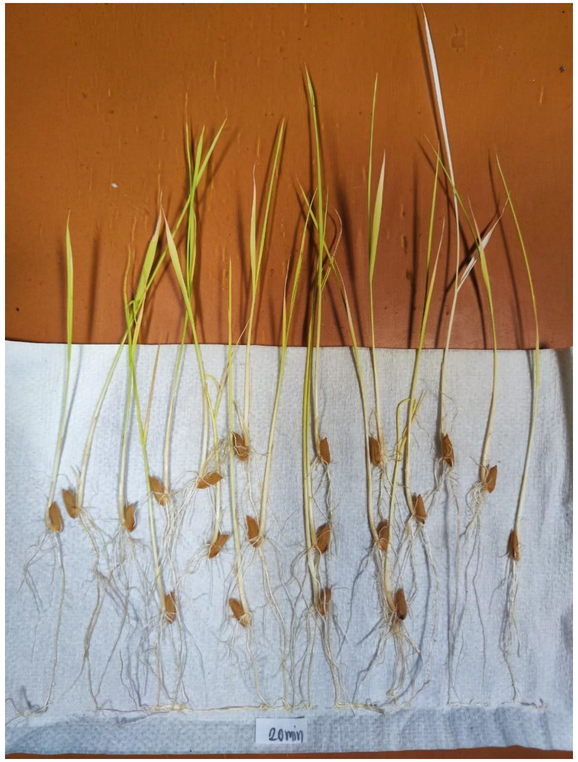
Rice germination at 14 day

Control



10 min

15 min



20 min

Results

Table 1 Growth characteristics of green oak.

Treatments[1]	Plant growth (42 days after planting)					
	Plant height (cm)	Leaf width (cm)	Canopy (cm)	No.leaves	Root length (cm)	SPAD
Hoagland	**19.42 a**	**14.50 a**	**29.17 a**	**14.50 a**	**24.57 a**	**21.98 b**
Hoagland (NO^-_3 = 0)	13.00 b	7.17 b	15.67 b	6.67 b	16.37 b	19.93 c
Plasma fertilizer	**18.00 a**	**14.75 a**	**28.33 a**	**17.00 a**	**18.55 b**	**25.43 a**
CV (%)	13.15	22.83	15.12	16.59	17.75	4.81
$LSD_{0.05}$	2.72	3.41	4.54	2.60	4.33	1.33

[1] Means in the same column followed by different letters are significantly different ($p \leq 0.05$) by LSD

SPAD = Leaves color intensity

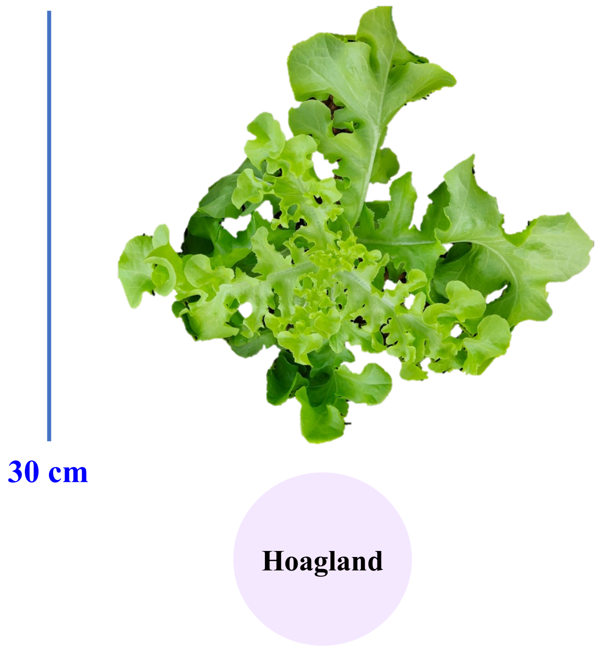
Leksakul, K., et al. (2021) Generating nitrate and nitrite on green oak lettuce in hydroponic farming by plasma system *Applied Engineering in Agriculture,* 37 (1)

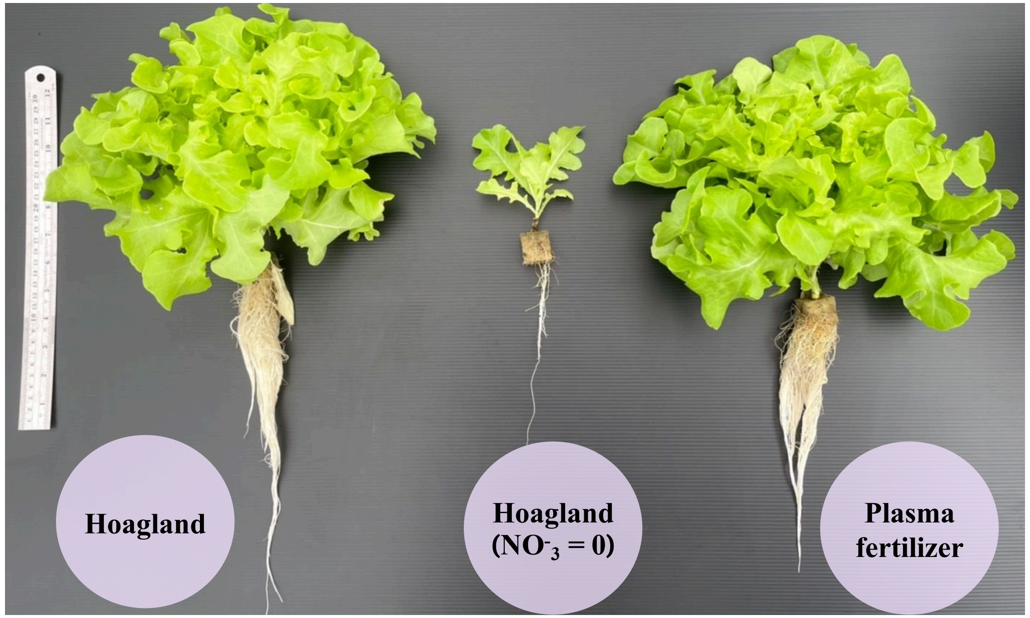
Results

Table 2 Photosynthesis rate and fresh weight of green oak.

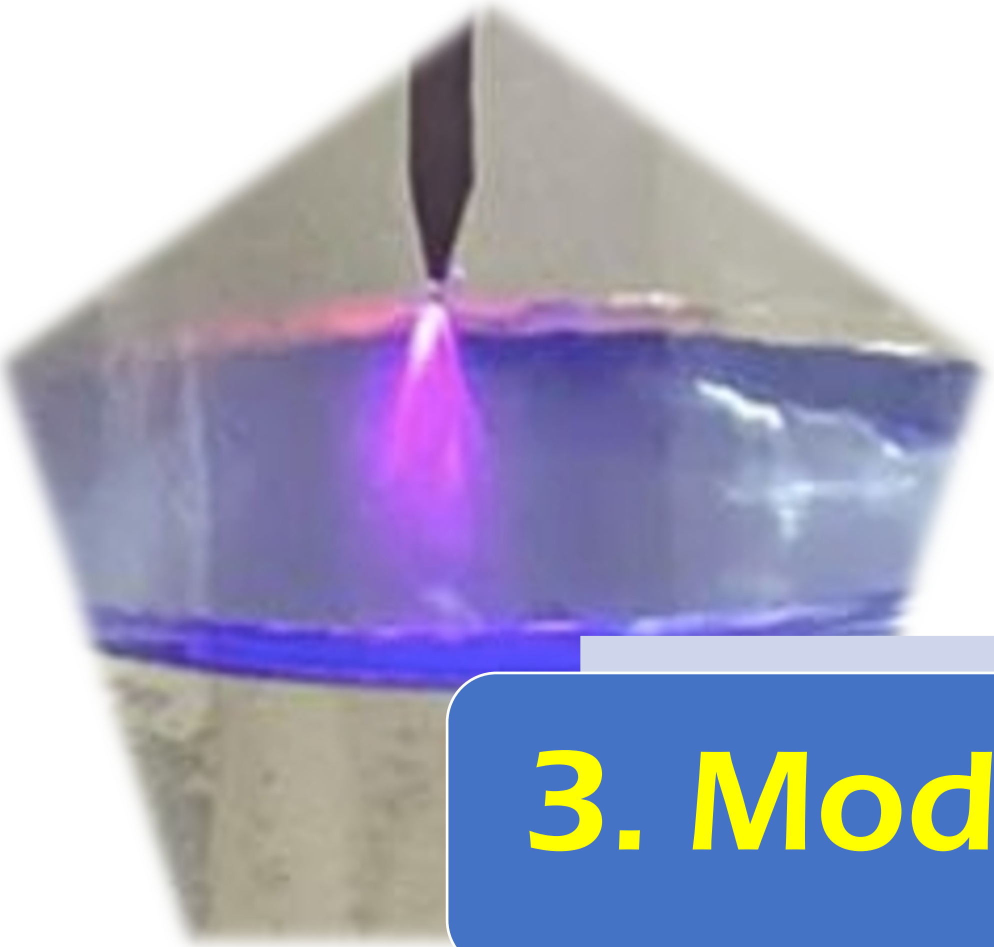
Treatments[1]	Harvesting stage		
	Photosynthesis rate ($\mu mol\ m^{-2}\ s^{-1}$)	**Fresh weight shoot (g)**	**Fresh weight root (g)**
Hoagland	**2.09 b**	**52.55 a**	**7.29 a**
Hoagland (NO^-_3 = 0)	0.72 c	6.29 b	1.34 b
Plasma fertilizer	**3.02 a**	**63.84 a**	**7.02 a**
CV (%)	33.67	37.74	46.92
$LSD_{0.05}$	0.80	18.99	3.01

[1] Means in the same column followed by different letters are significantly different ($p \leq 0.05$) by LSD

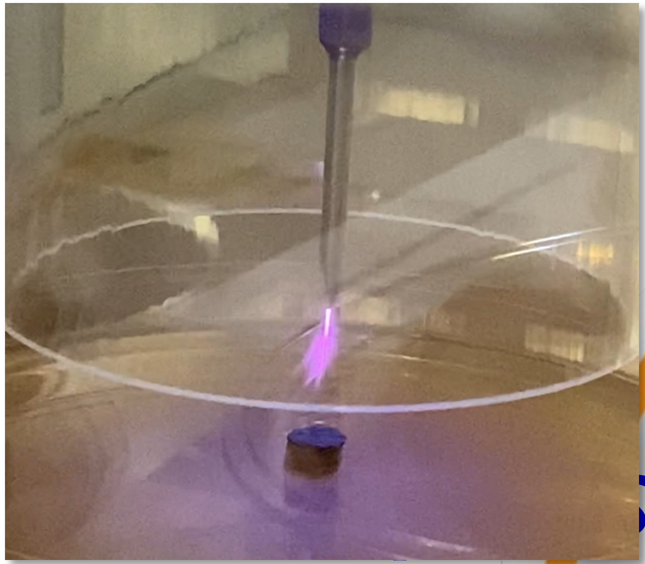




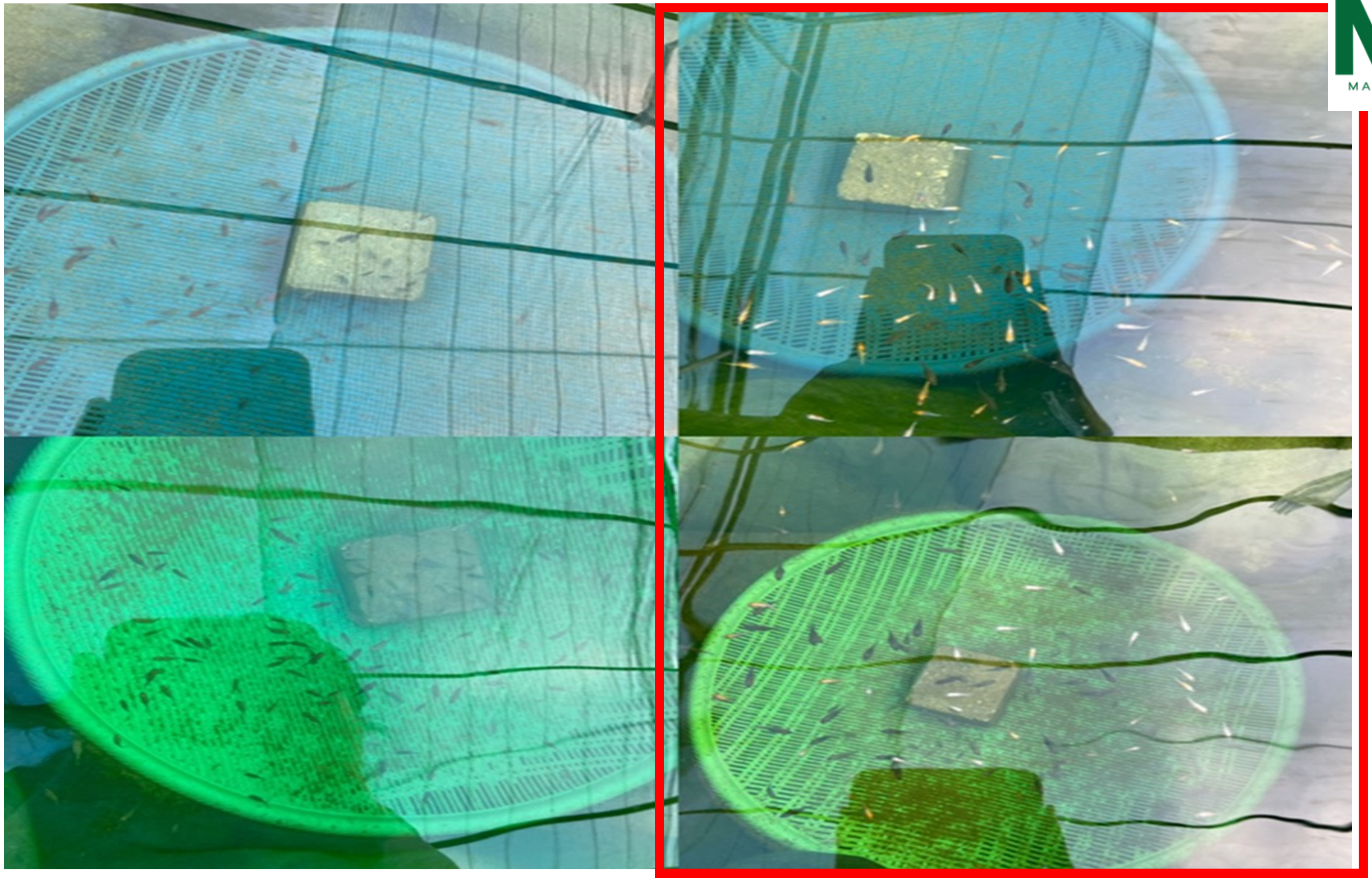

PAW-Foliar spray
Control

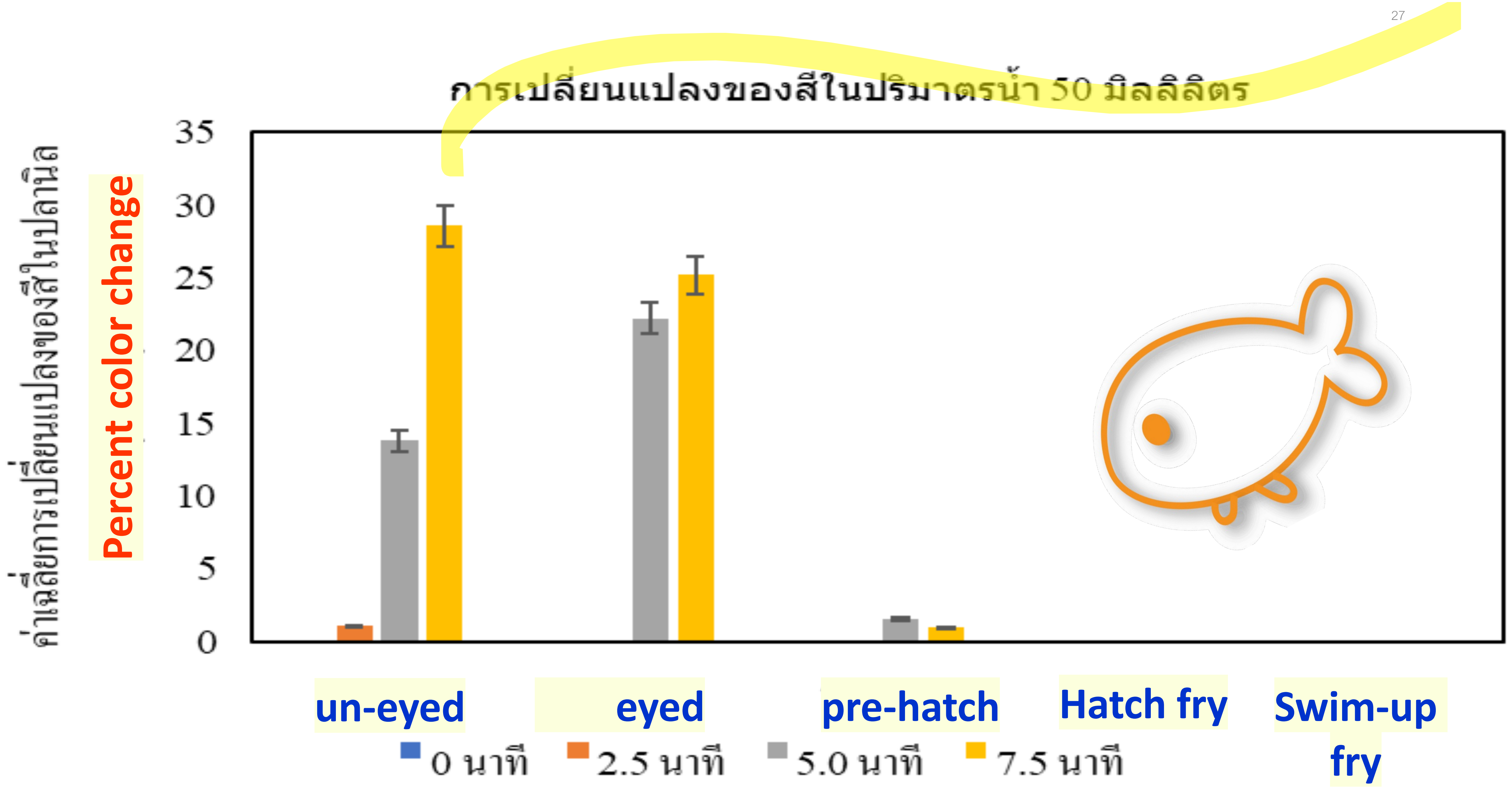


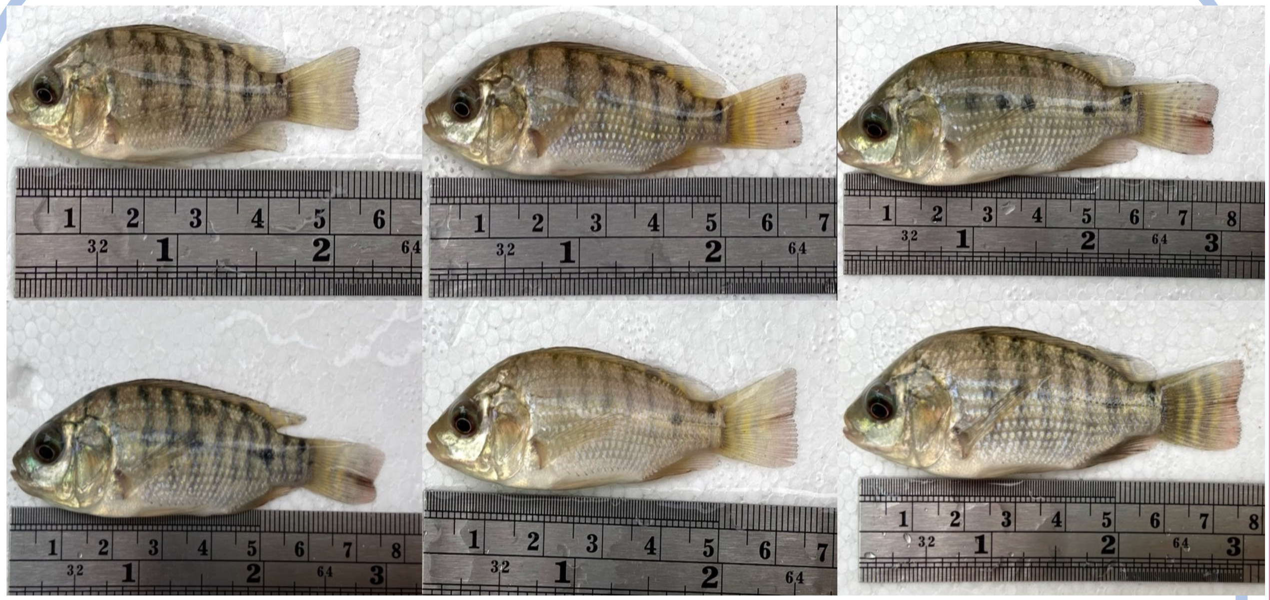
3. Moderate Biocatalyst



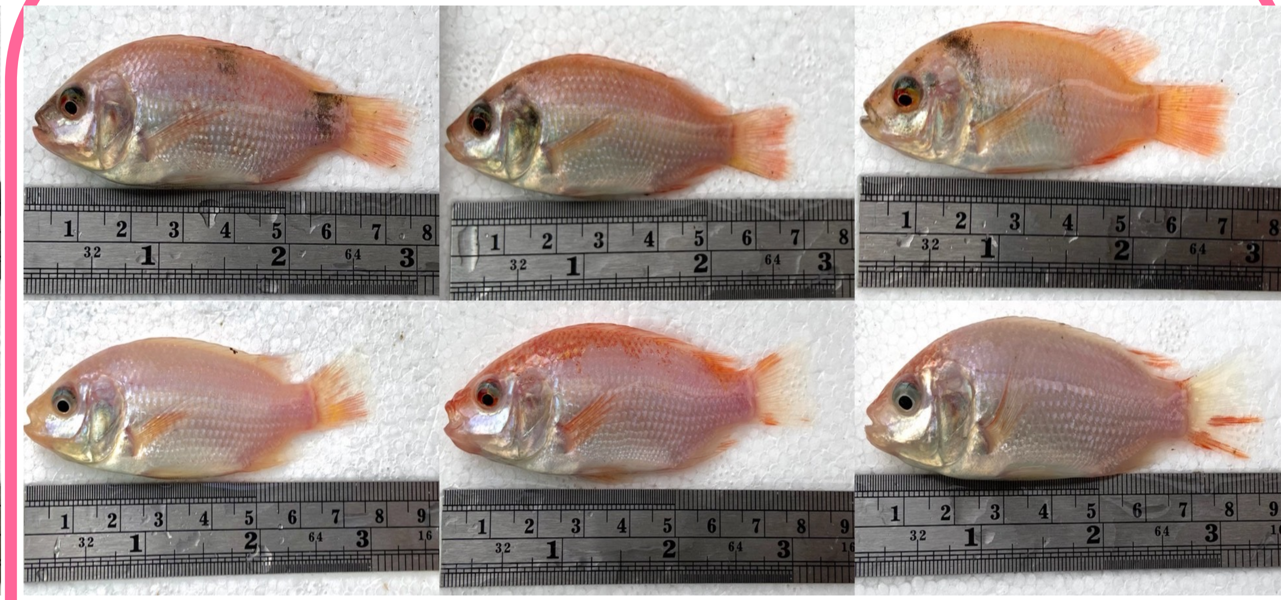
study of color changing in Nile Tilapia (*Oreochromis niloticus*) Induced by Plasma activated water




การเปลี่ยนแปลงของสีในปริมาตรน้ำ 50 มิลลิลิตร
ค่าเฉลี่ยการเปลี่ยนแปลงของสีในปลานิล
Percent color change
35
30
25
20
15
10
5
0
un-eyed
eyed
pre-hatch
Hatch fry
Swim-up fry
0 นาที
2.5 นาที
5.0 นาที
7.5 นาที



Control

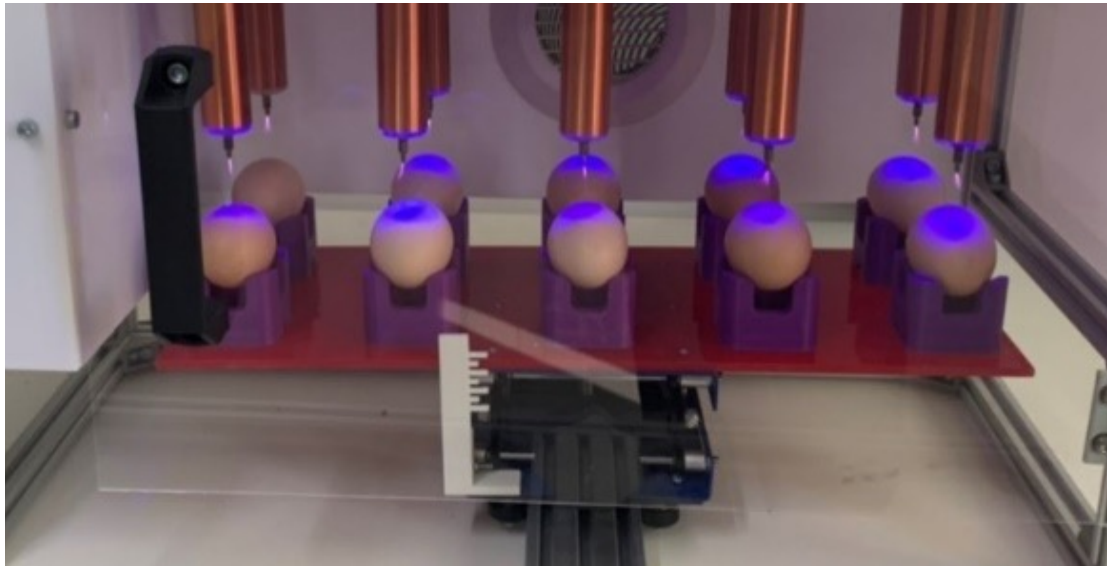


PAM-induced

Treatments

Treatment	Plasma	No. HE
T1	control	264
T2	10s	264
T3	20s	264
T4	30s	264
Total		1,056

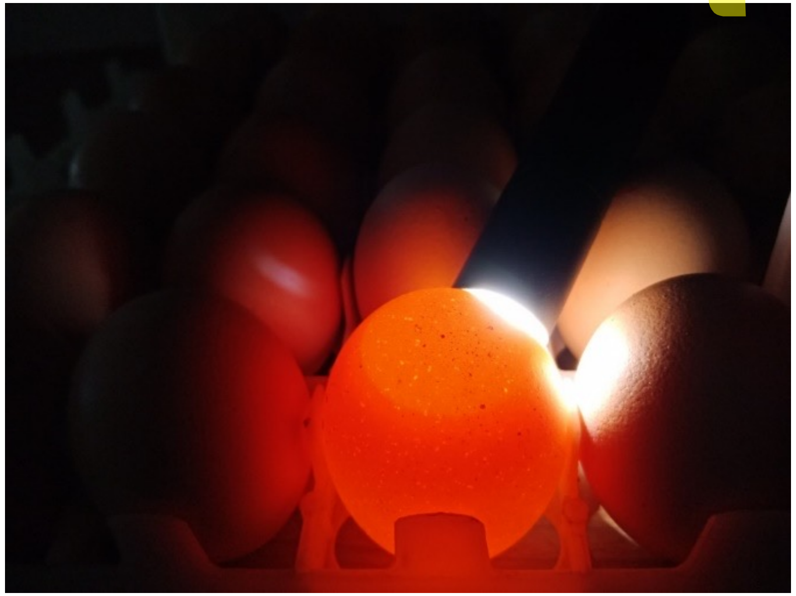
Breed: crossbred native chickens Pradu Hang Dam
Age of hatching egg 36/14 wp.



plasma exposed on day 4th of incubation

Sakulthai, et al.(2023) Improving the efficiency of crossbred Pradu Hang Dam chicken production for meat consumption using cold plasma technology on eggs. *Scientific Reports* 13, 2836

Candling day 18 of incubation



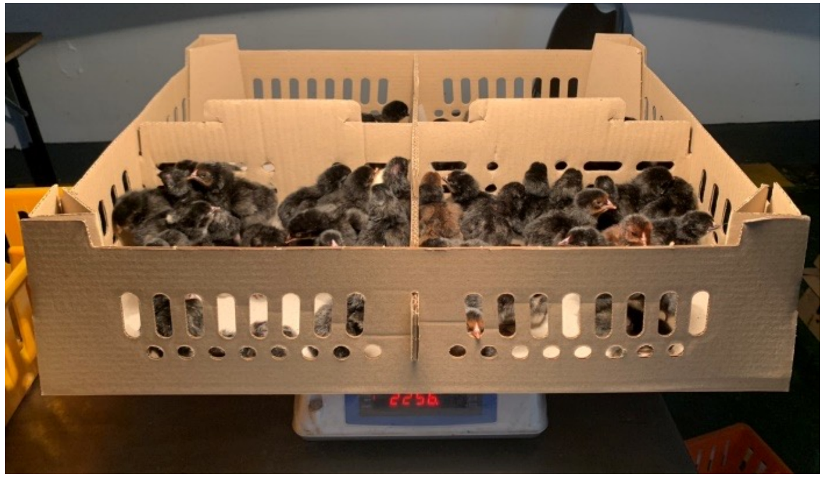
Infertile egg

Early-mid-late dead egg

Fertile egg

Data collection

- %Hatchability
- Day old chick weight
- Shank length



Ratio male and female

Treatment	Plasma	ratio male	ratio female
T1	Control	51.05	48.95
T2	10s	48.30	51.70
T3	20s	54.79	45.21
T4	30s	58.67	41.33
Avg.		53.20	46.80

Growth performance

Weight gain

		0-7 day	0-14 day	0-21 day	0-28 day	0-35 day	0-42 day
Male	T1	53.00	152.00	268.00	418.00	558.00	750.00
	T2	52.33	152.33	275.33	432.33	595.33	794.33
	T3	52.00	153.00	282.00	429.00	586.00	813.00
	T4	52.33	151.33	277.33	426.33	576.33	778.33
Female	T1	50.67	139.67	237.67	366.67	494.67	652.67
	T2	50.67	140.67	239.67	372.67	506.67	681.67
	T3	50.79	138.79	242.79	365.79	494.79	666.79
	T4	50.85	135.85	236.85	362.85	479.85	645.85

Daily gain

		0-7 day	0-14 day	0-21 day	0-28 day	0-35 day	0-42 day
Male	T1	7.57	10.86	12.76	14.93	15.94	17.86
	T2	7.48	10.88	13.11	15.44	17.01	18.91
	T3	7.43	10.93	13.43	15.32	16.74	19.36
	T4	7.48	10.81	13.21	15.23	16.47	18.53
Female	T1	7.24	9.98	11.32	13.10	14.13	15.54
	T2	7.24	10.05	11.41	13.31	14.48	16.23
	T3	7.26	9.91	11.56	13.06	14.14	15.88
	T4	7.26	9.70	11.28	12.96	13.71	15.38

Growth performance

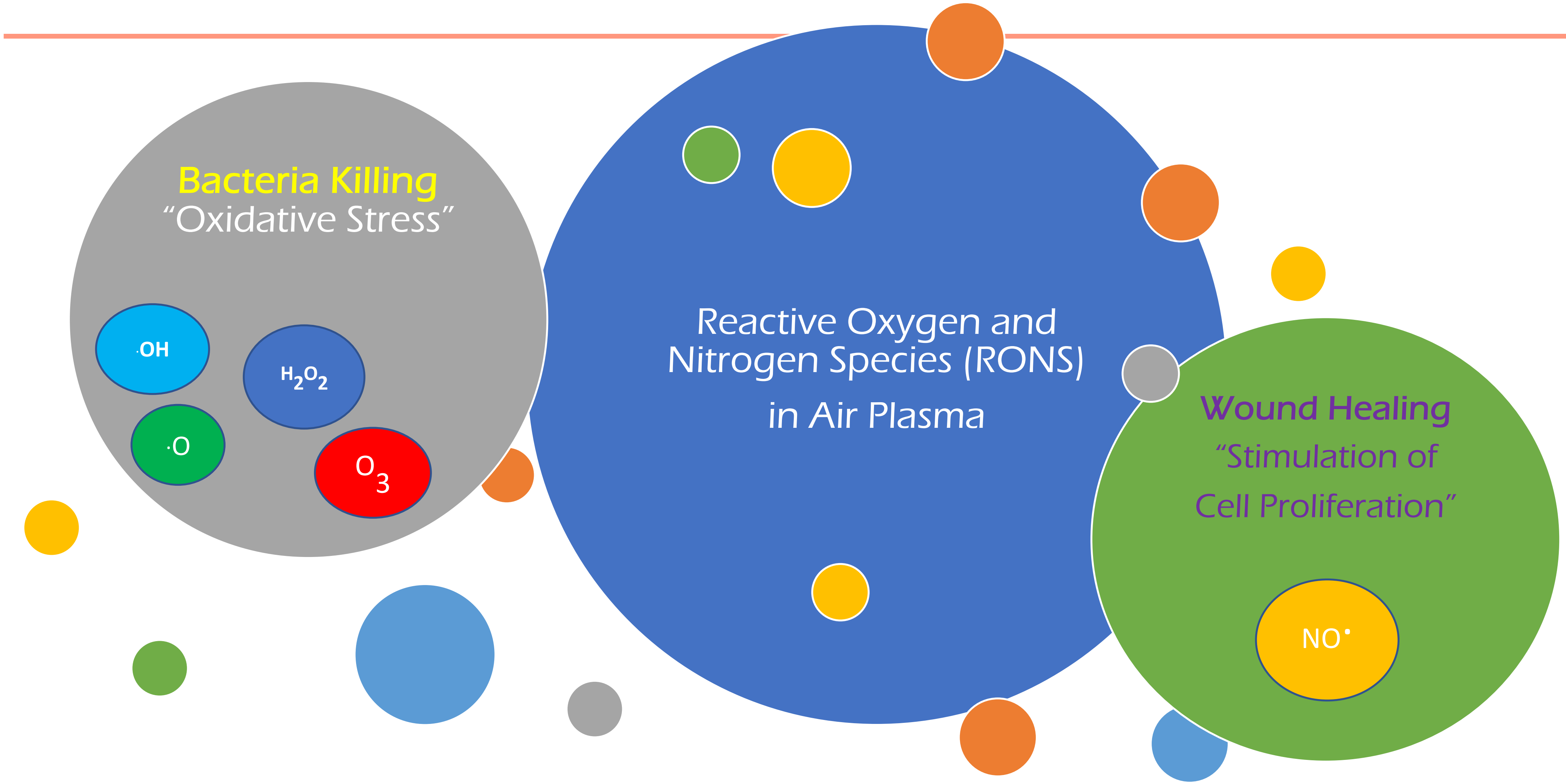
14 days

Male

Female

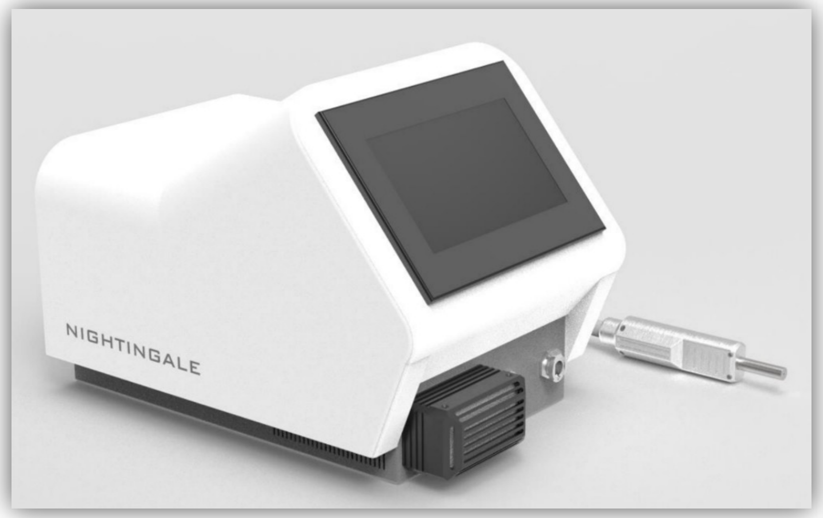
42 days

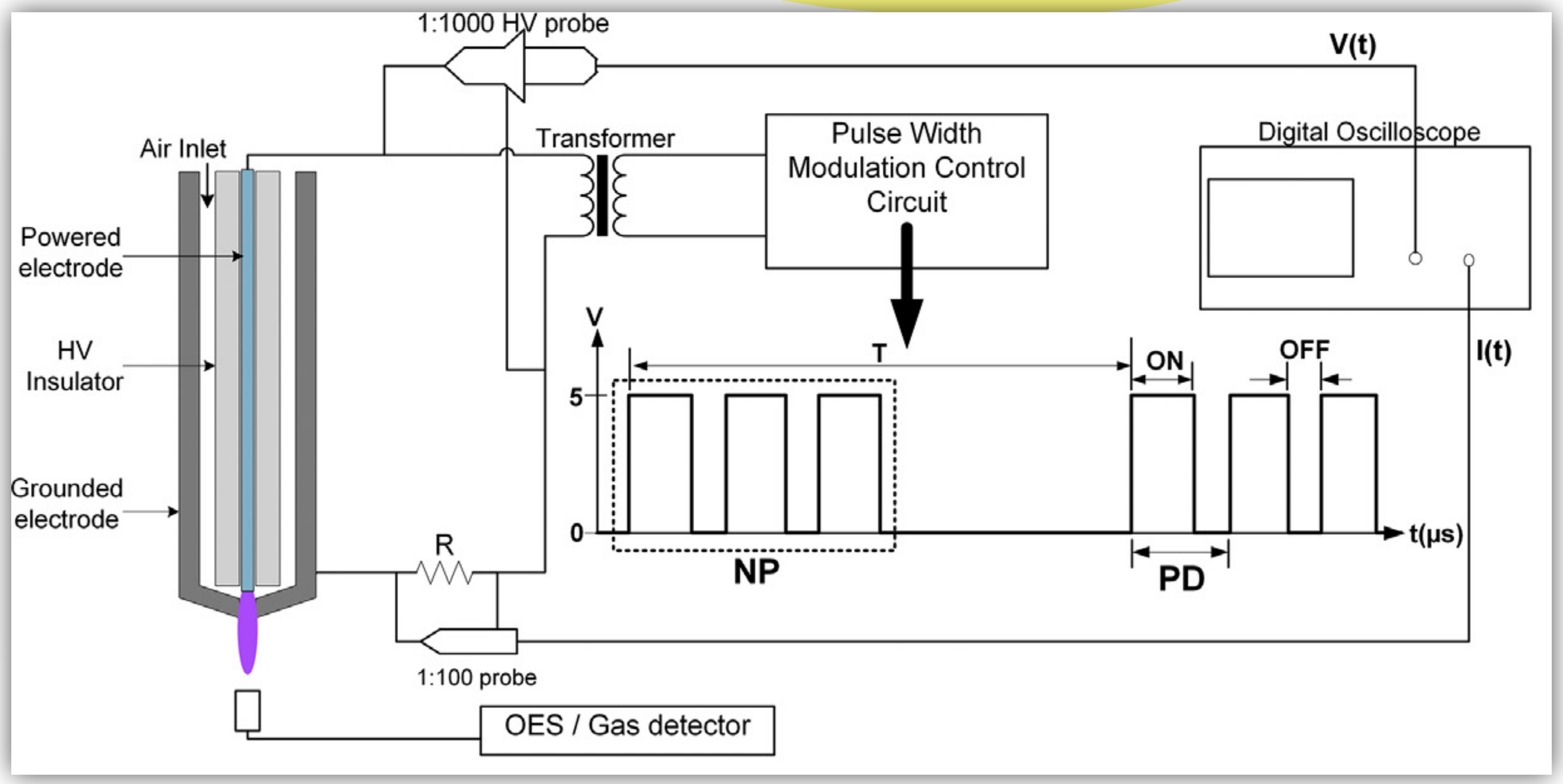
4. Cold Plasma in Medicine



Air Plasma Jet Device

"Nightingale®"





Applications

- Wound healing[1]
- Tissue Culture[2]
- Cancer therapy

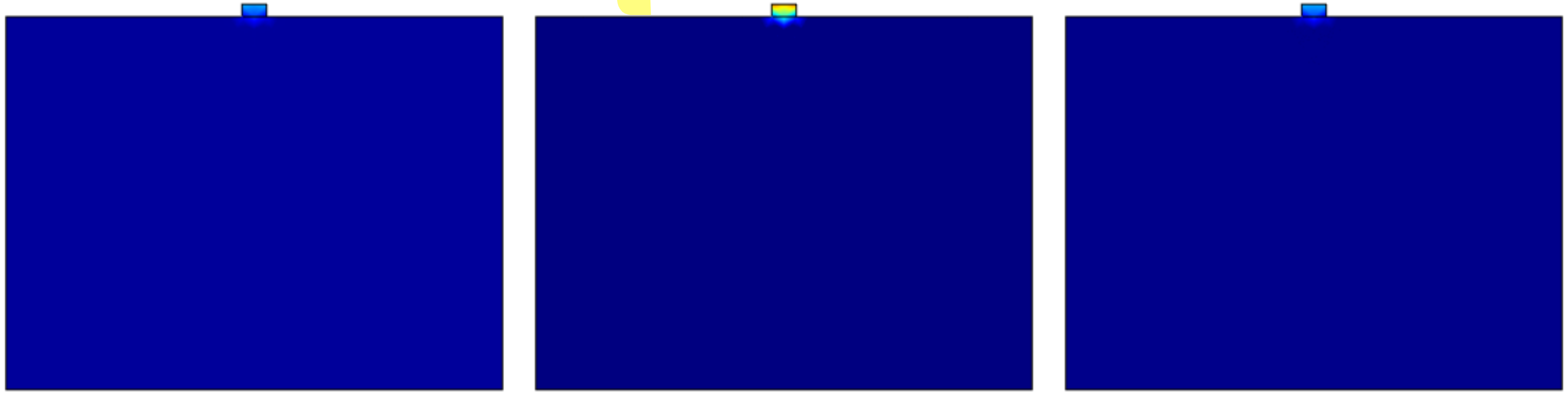
DC pulse in burst mode with 2-kHz repetition rate, voltage 2 kV, number of pulses (NP) at 4, 7, and 10, pulse width (PW) 1 us at 10 us pulse delay (PD) and flow rate of air 5 l/min (plasma dissipated power at 0.28, 0.43 and 0.62 W).

[1] *Thana et al., 2019.*
[2] *Kosumsupamala et al., 2022*
http://innoplascm.com

Radicals flow simulation

Species dynamics

- 20 mS / 15 mm



O_3 NO_2 NO

InnoPlasCM

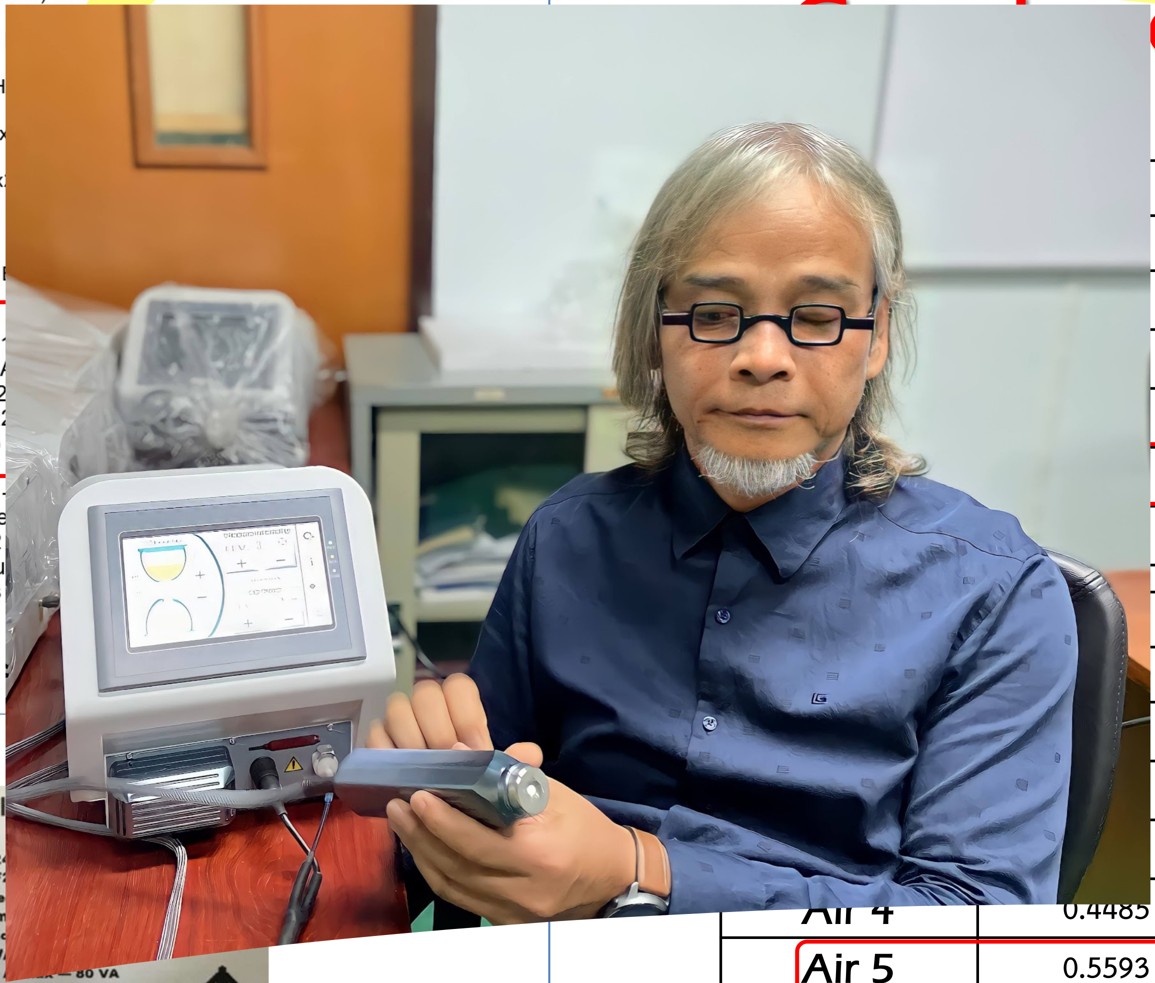
TECHNICAL FEATURES

Input Voltage	220-240 Vac 50-60 Hz.
Output RF Frequency	Pulsed at 1uSec - 93 KHz Modulation
HF transfer mode	Capacitive – Not in contact
Output power	< 50 VA (Watt) Max
Air Flow	11L/min -
Power line absorption	0,4A (50H
Line protection Fuse	2 Fuse 5x
Internal protections	1 Fuse.5x
Activation command	TIMED
Class protection and Type	I – TYPE

Manufacturer Certification : ISO 9001: 2015 EN 1
Reference Standard : EN 60601-1: 2006 / A
EN 62304:2006/A1:2
IEC 62304:2006/A1:2
EN 60601-1-2: 2015

Included (Standard CEI 62-39 - Class. CEI 62-39 - tion - Electrical appliances for aesthetic use. Gene ard CEI EN 60601-2-3 - Class. CEI 62-14 - CT 62 Medical electrical equipment - Part 2: Particular ru vices + VARIANT: CEI EN 60601-2-3 / A1 - Class 1999 and related variants.)

TECHNICAL LABEL



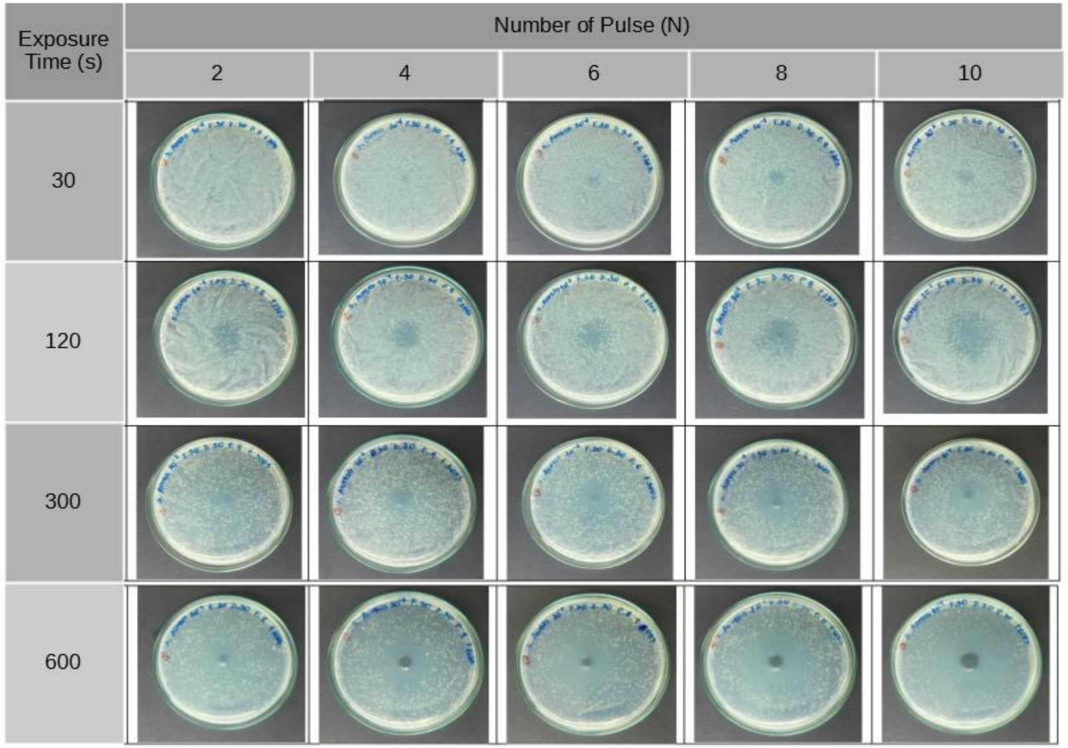
C... e properties

	Nitric Oxide (ppm)		
	1	Power 2	Power 3
		24.39	38.78
		24.49	42.11
		26.07	41.55
		26.28	44.15
		23.73	37.10

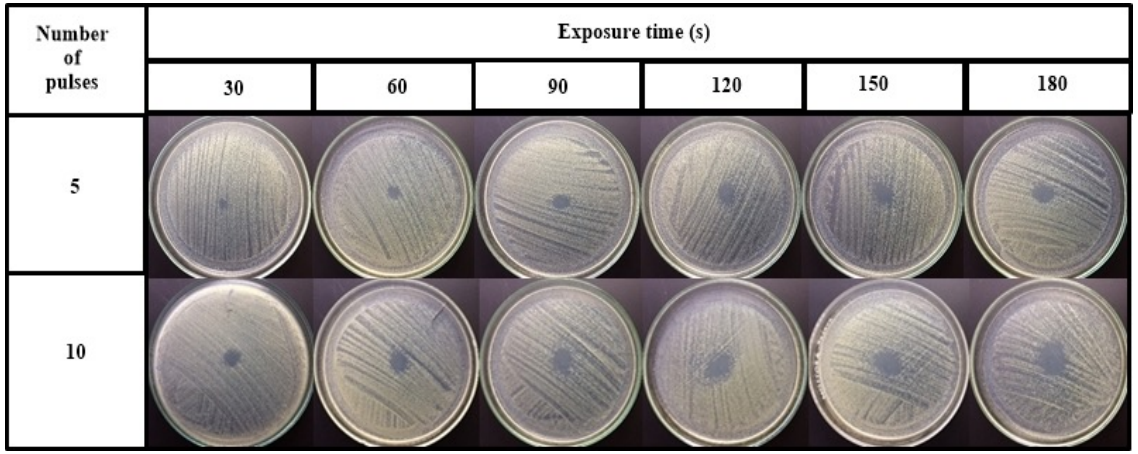
	Ozone (ppm)		
	1	Power 2	Power 3
		0.0404	0.0421
		0.0950	0.0631
		0.1936	0.1275
Air 4	0.4485	0.3484	0.2726
Air 5	0.5593	0.5392	0.4871

* Measured at 1 cm distance

Bacteria inactivation



S. Aureus



Methicillin-resistant S. Aureus
(MRSA)

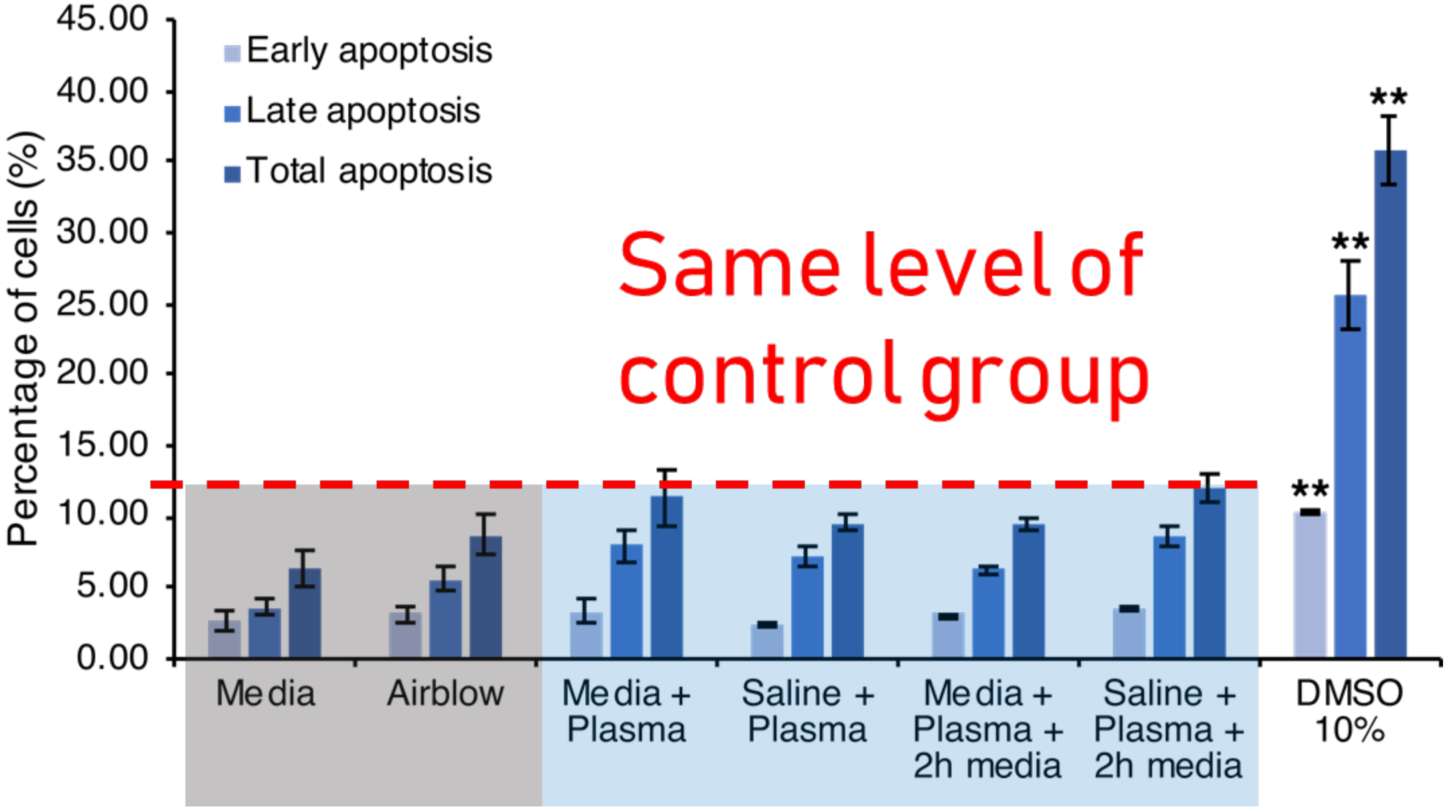
Thana P., et al. A compact pulse-modulation air plasma jet for the inactivation of chronic wound bacteria: Bactericidal effects & host safety (2020) *Surface and Coatings Technology*, 400, art. no. 126229,

II : Cell cytotoxicity

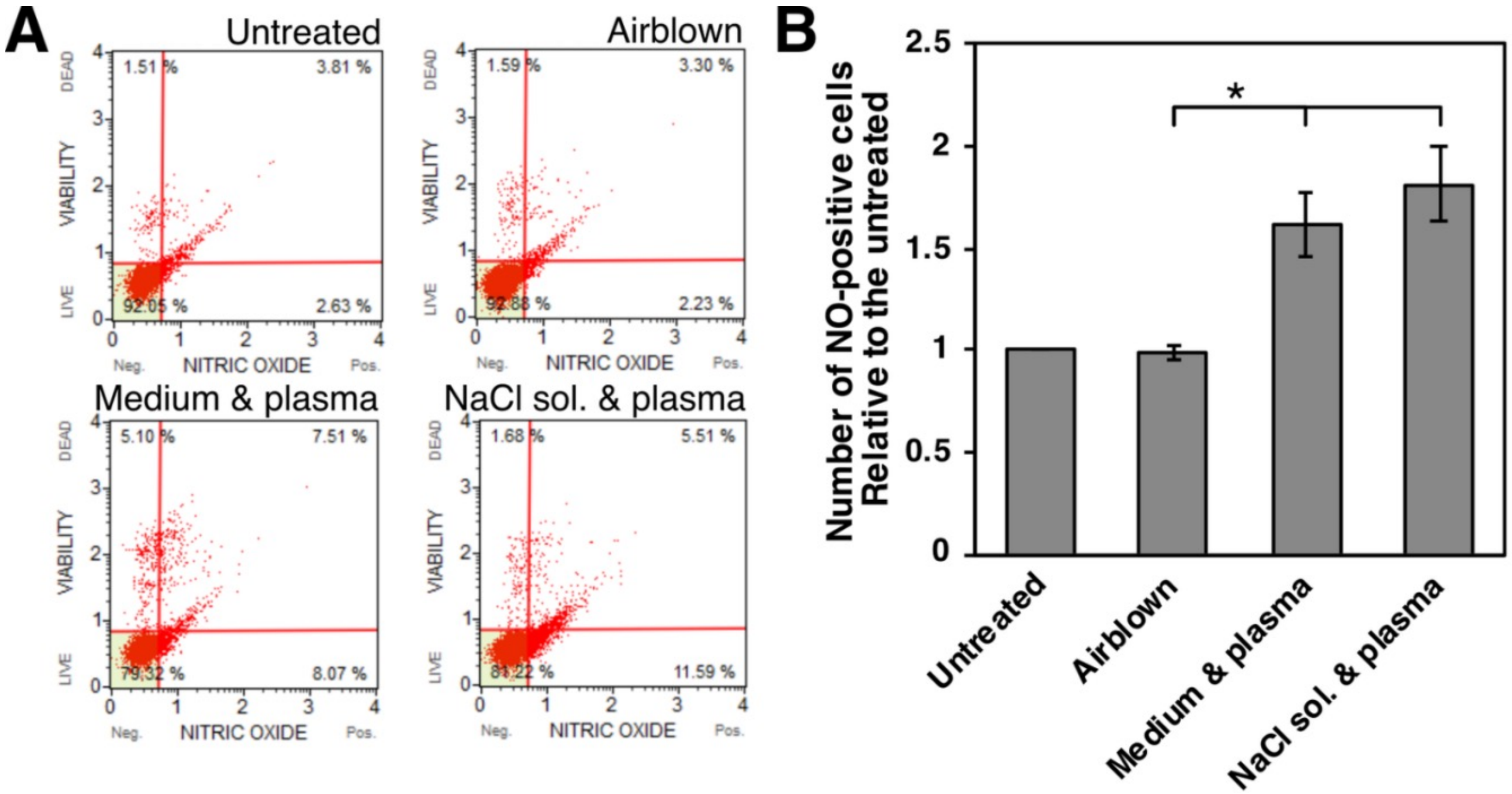
The apoptotic induction of HDF cells

To examine the apoptotic induction after plasma treatment.

The derived percentage of cells showed no significant induction on any states of apoptosis or necrosis. For the controls and the plasma-treated samples, the percentages of early, late and total apoptotic cells were 2-3%, 4-10% 6-11%, respectively. While, 10% of DMSO was used as a positive control of apoptosis induction.

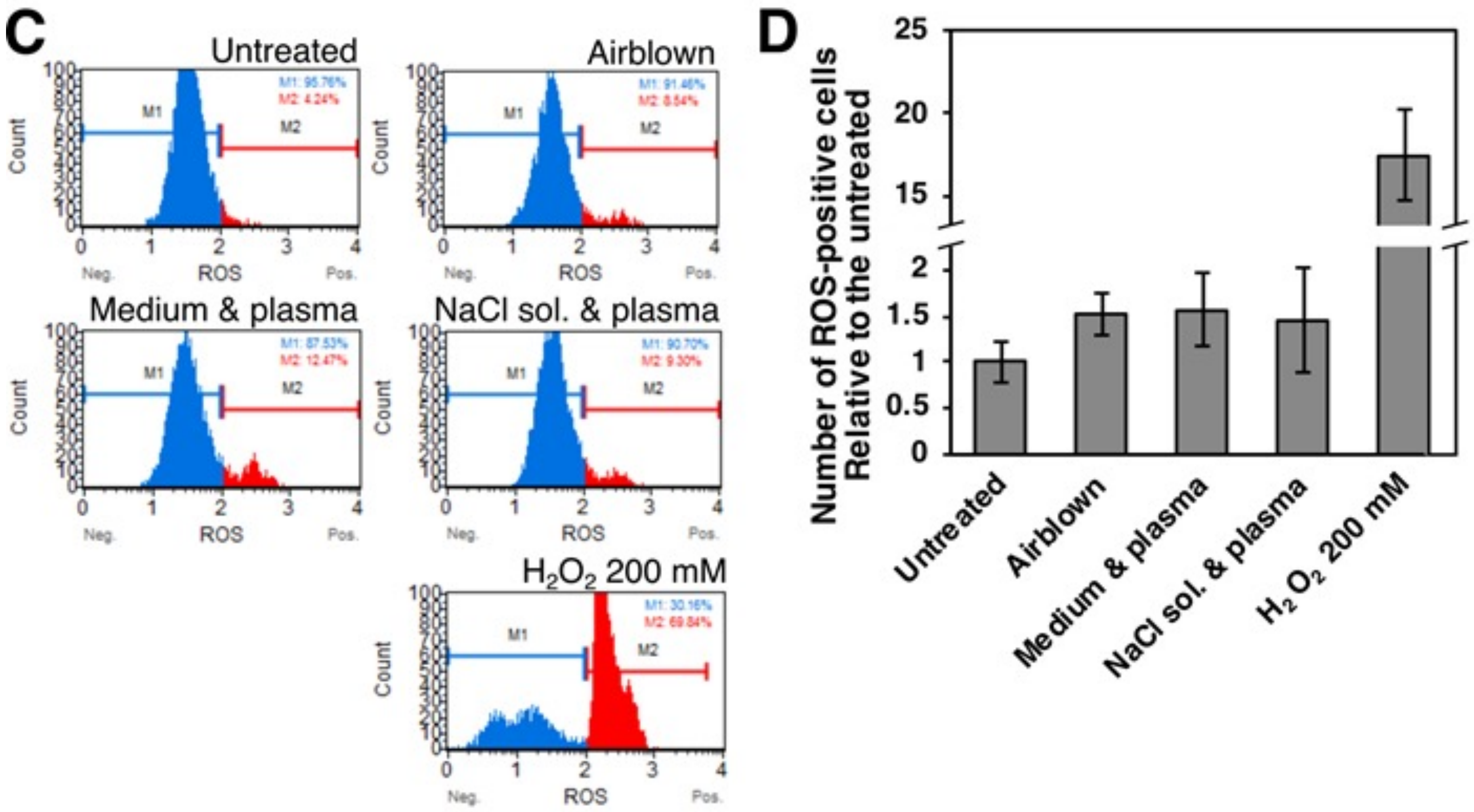


II : Cell cytotoxicity
The intracellular nitric oxide level in HDF cells



Air plasma jet increased intracellular nitric oxide level.

II : Cell cytotoxicity
The reactive oxygen species in HDF cells



But Air plasma jet not induced reactive oxygen species in HDF cells.

Lately Clinical Trial

60 days fully recovered/healed

- twice a week

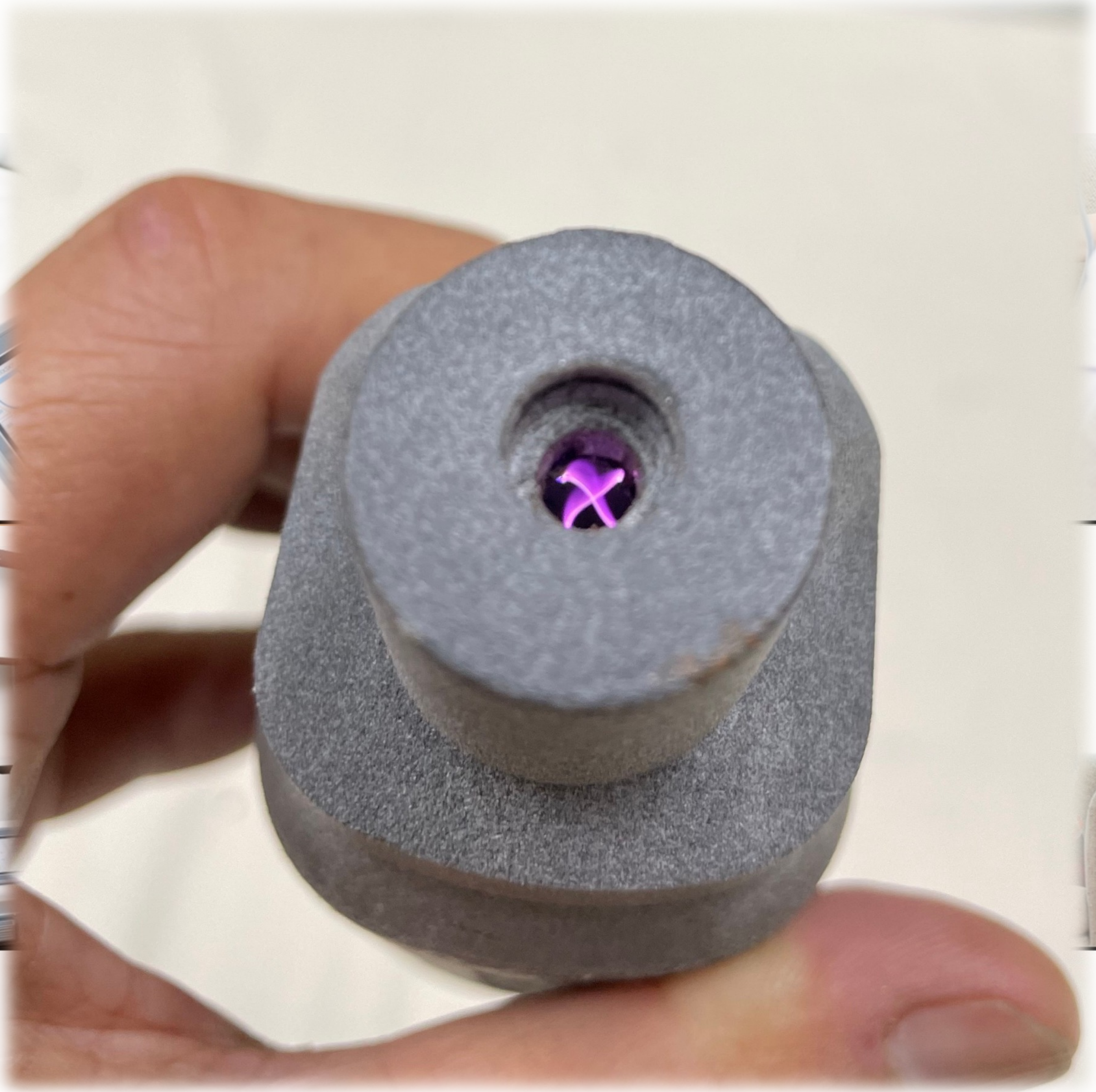
Lately Clinical Trial

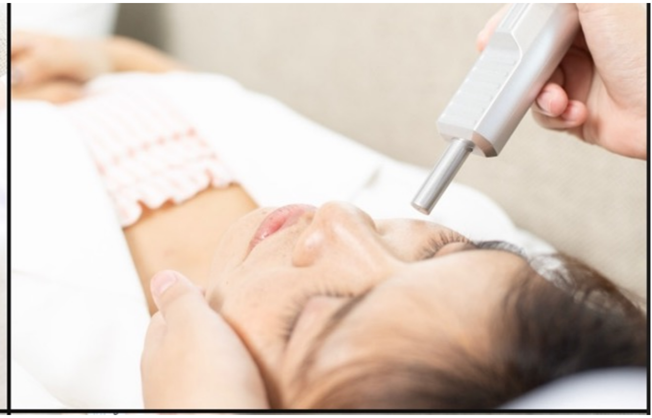
6 months : Healed

50 days fully recovered/healed

Vet Clinical Trial

Vet Clinical Trial





Conclusion & Perspectives

- The amounts of plasma-produced radicals can be controlled by varying the pulse width, pulse repetition rate, plasma generation time, and plasma gas composition.
- Proper dose for each level of bio-catalysis to be intensively studied:
  in-vitro/in-vivo.

For medicine, the human security/assessment concern:

- Curious study at the biomolecular level to be carried out/ continued.
- Also, cold plasma processing could be adopted/scaled up for near-future applications.

THANK YOU
FOR YOUR ATTENTION