Argon plasma *vs.* Air plasma: Characterization and Interaction with Biological Systems

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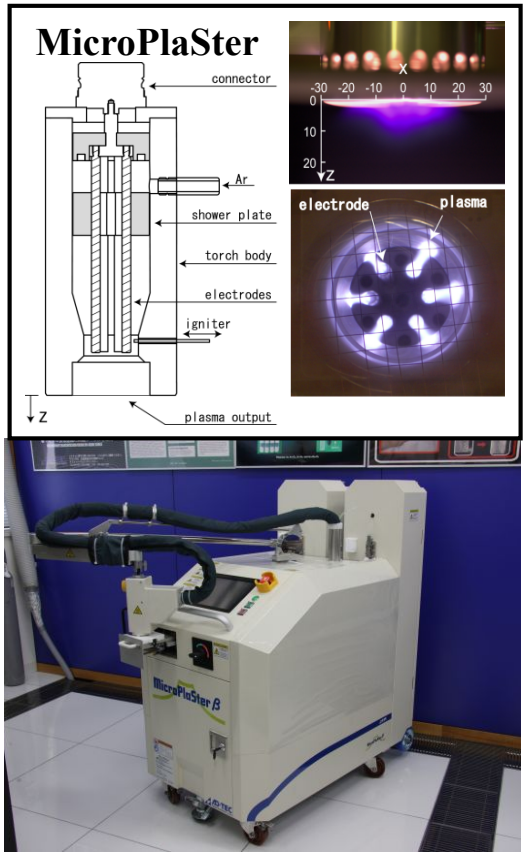
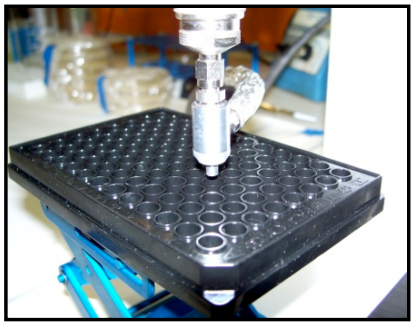
Plasma Devices

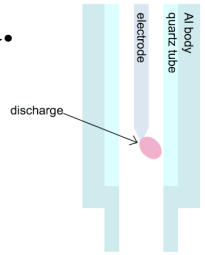
Ar plasma driven by microwave



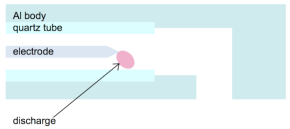
MiniPlaSter



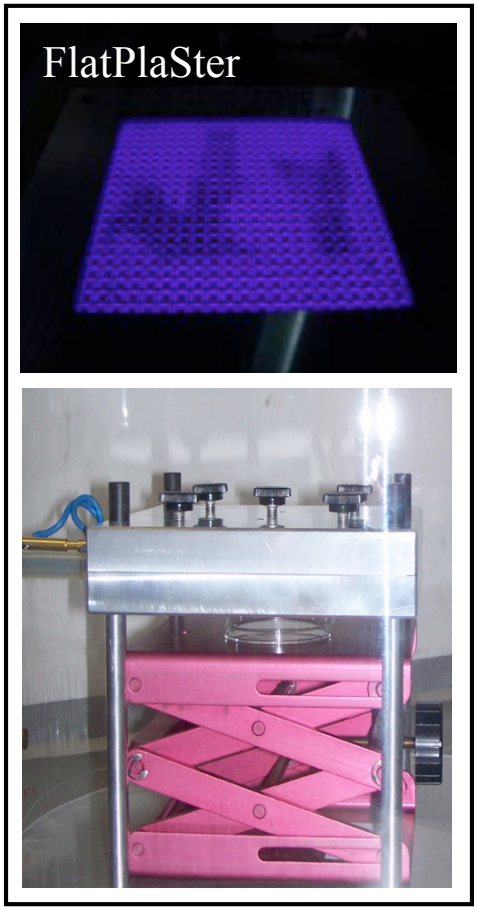
a.



b.



Air plasma (surface micro-discharge)

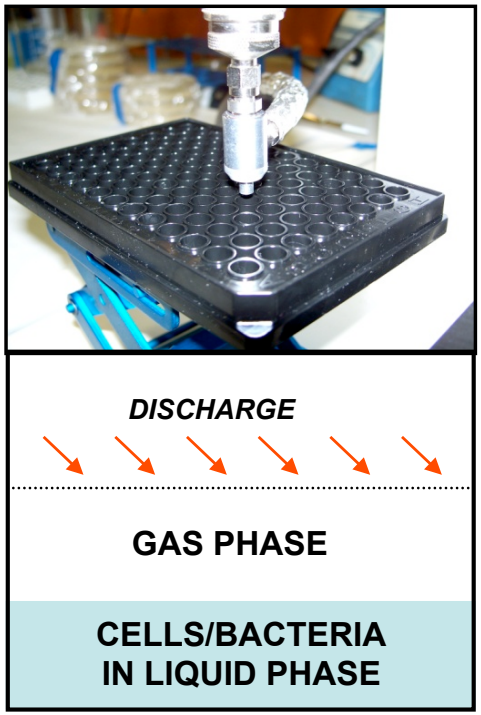


Study Goal

Optimizing plasma for chronic wound disinfection

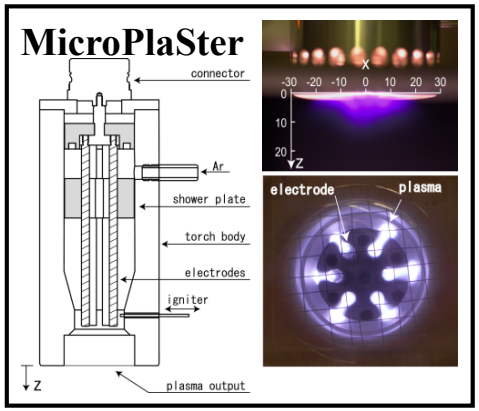
- Assessing relative bactericidal contribution of different components of argon and air plasmas
- Assessing effects of different plasma components on human cells

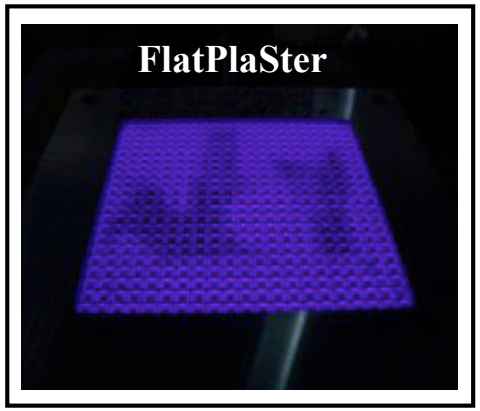
Biologically Active Components of Plasmas



- UV Radiation produced by excited state molecules and atoms (direct effect on cells and bacteria)
- Reactive Species and intermediate products of their reaction with liquids
  - Reactive Nitrogen and Oxygen Species (NO, NO_2, $ONOO^{\cdot}$, $O_2^{\cdot}$, O_3, $^{\cdot}OH$) diffused from the gas phase
  - Reactive species produced by photoionization of liquids (*e. g.* H^+, ^{-}OH, H_2O_2)
  - Reactive species produced by interaction of ions and excited state molecules and atoms with liquds (*e. g.* H^+, ^{-}OH, H_2O_2)

Biologically Active Components of Plasmas (overview)

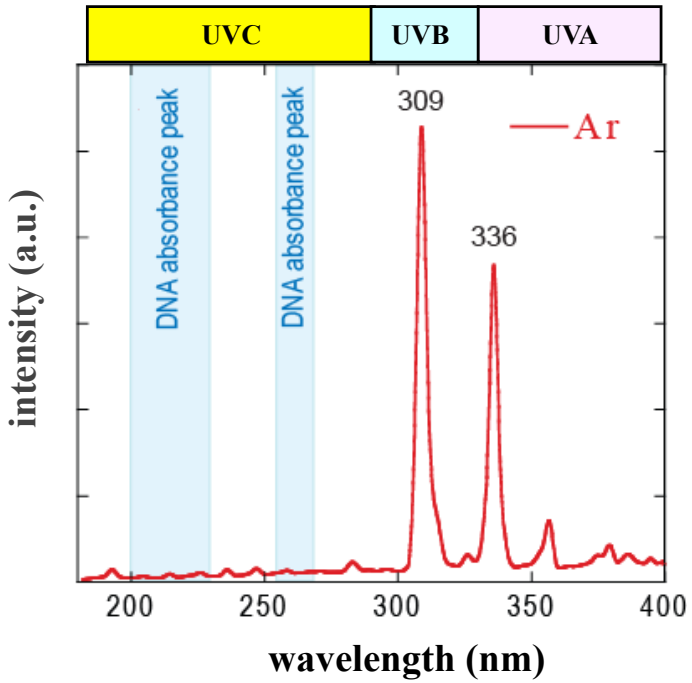




Plasma factors and their effects on cells/ bacteria	Argon plasma *Power 60 W ; 1.2 sccm Ar flow; distance from the torch 20 mm*	Air plasma *9 kV, 10 kHz; no gas flow; distance from the device 20 mm*
Direct effect of UV	↘ 30 μW/cm^2	<0.8 μW/cm^2
RS produced *via* liquid photoionization	↘ ~ 10 μM H_2O_2 (10 min irradiation)	no detectalbe H_2O_2 (10 min irradiation)
ROS and RNS diffusion from the gas phase	↘ 8 μM NO_2^-/NO_3^- (10 min irradiation)	↘ > 300 μM NO_2^-/NO_3^- (10 min irradiation)
RS produced *via* liquid ionization by ions and excited molecules and atoms	↘ Measured only for MiniPlaSter	no detectalbe H_2O_2 (10 min irradiation)

UV Radiation Produced by Ar Plasma

Optical spectrum of Ar plasma-generated UV



Power density of the UV radiation produced by MicroPlaSter

(microwave power 60 W; Ar flow 1300 sccm; 20 mm distance between the torch and object)

	UV power density ($\mu W/cm^2$)	UV power / 5 min of irradiation (mJ/cm^2)
Total UV	30.0	9.0
Short wavelength UV (200-260 nm)	4.7	1.4

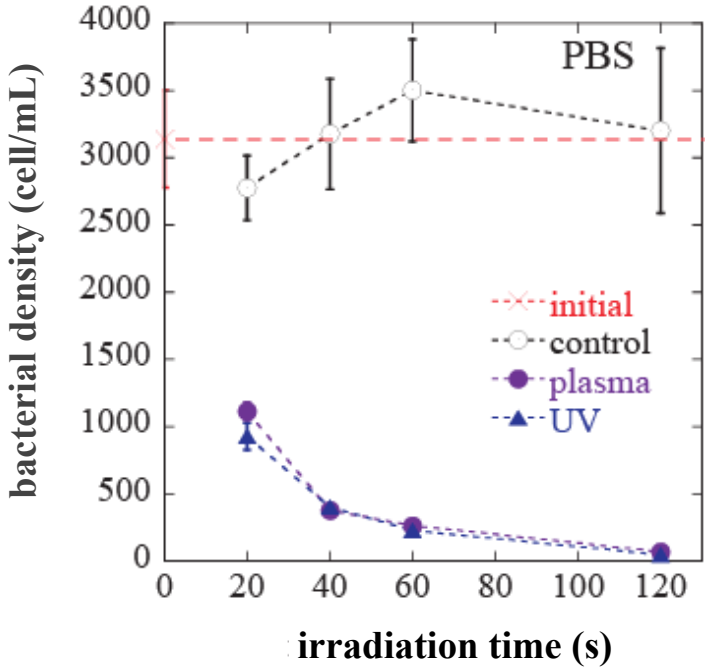
Argon plasma produces polychromatic UV radiation with two main peaks in the UVB and UVA ranges

UV

UV Radiation Produced by Ar Plasma

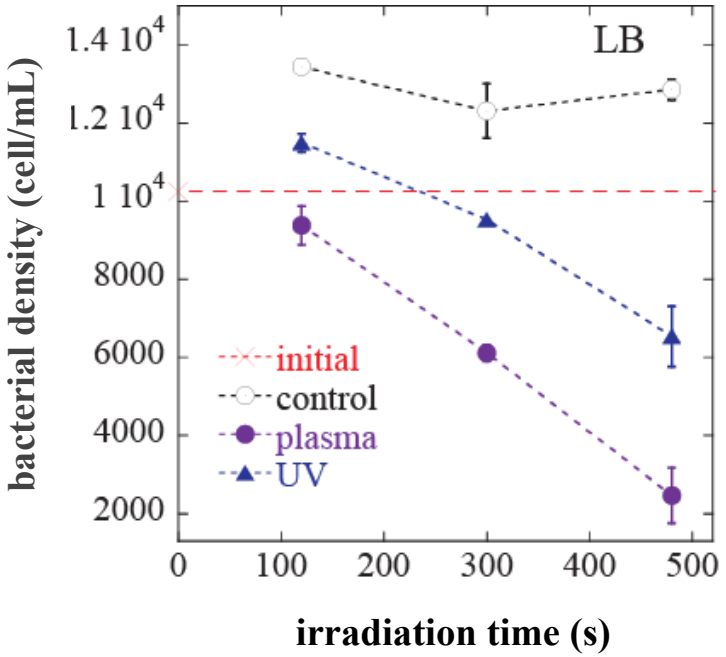
Effects on Bacteria

Plasma sterilization of clear solutions
(with MiniPlaSter)



- UV is the main short-term sterilizing factor of argon plasma
- 1.5 mJ/cm^2 of Ar plasma-generated UV causes 100% bacterial density reduction in a clear saline solution

Plasma sterilization of nontransparent organic solutions
(with MiniPlaSter)



- 100% of bacterial density reduction in the LB medium is achieved by 10 min of plasma treatment
- Contribution of plasma-generated UV to the LB sterilization is ~ 50%

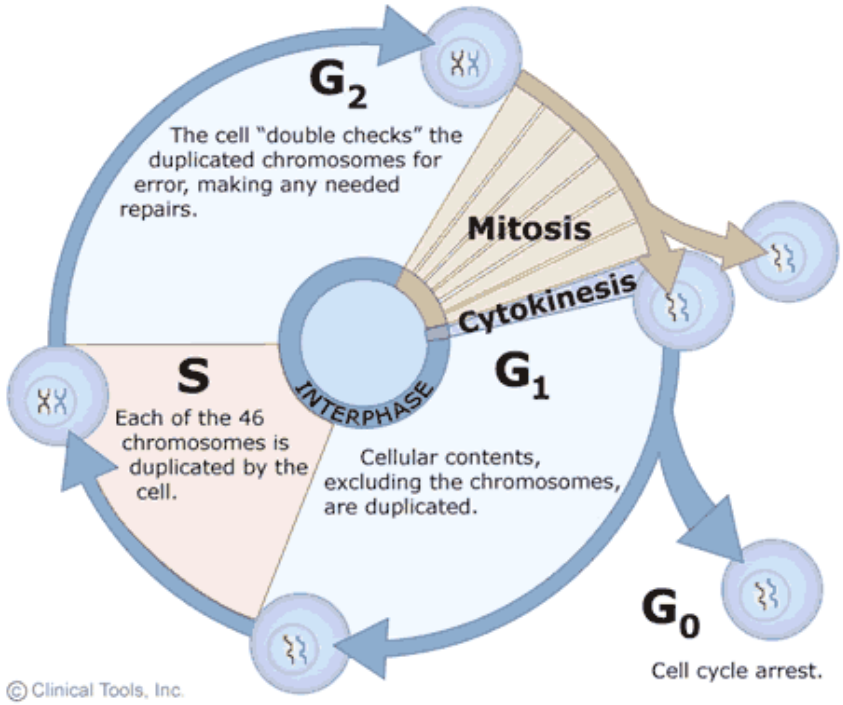
UV

Effect of UV Radiation on Human Cells

Necrosis - accidental cells death

Apoptosis - programmed cell death

Cell cycle arrest - the process by which the cell cycle is halted during one of the normal phases (G1, S, G2, M)

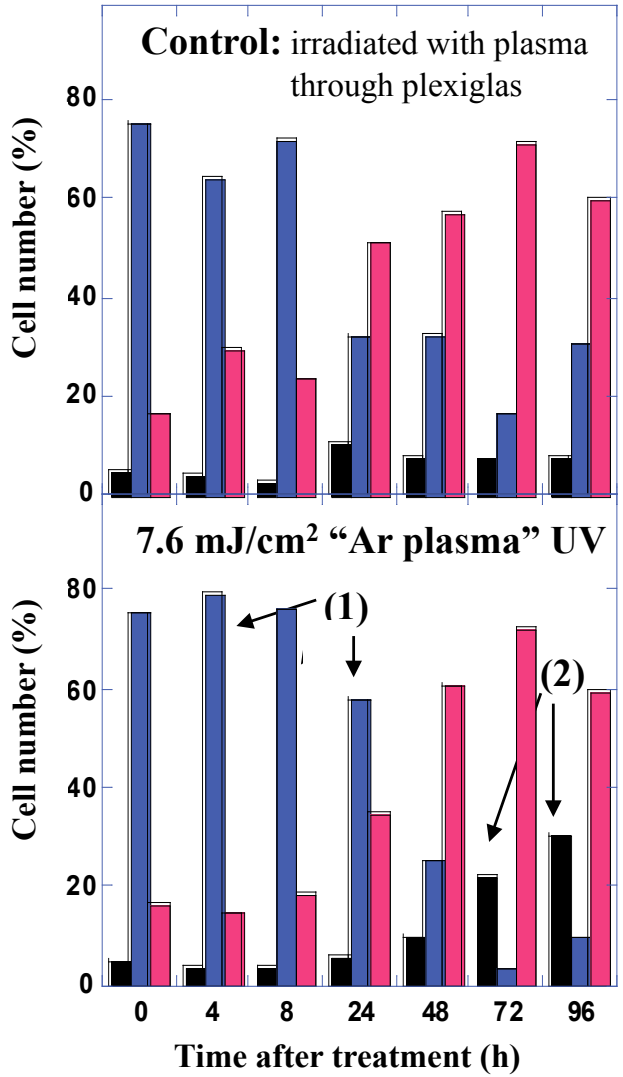


Cell cycle Phases

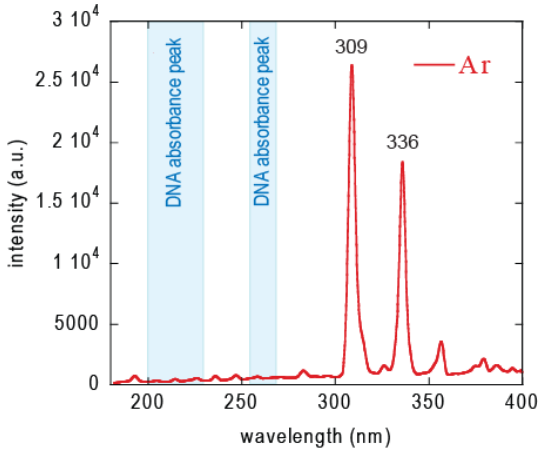
UV

UV Radiation Produced by Ar Plasma

Effects on Human Skin Fibroblasts



- **Apoptotic cells**
- **Non-dividing cells (G1 phase)**
- **Dividing cells (S/G2 phases)**



7.6 mJ/cm² is the pro-apoptotic limit of total UV radiation produced by Ar plasma (4 min of irradiation with MicroPlaSter in clinics)

(1) Temporary inhibition of fibroblast proliferation (G1 arrest) (see 24 hr)

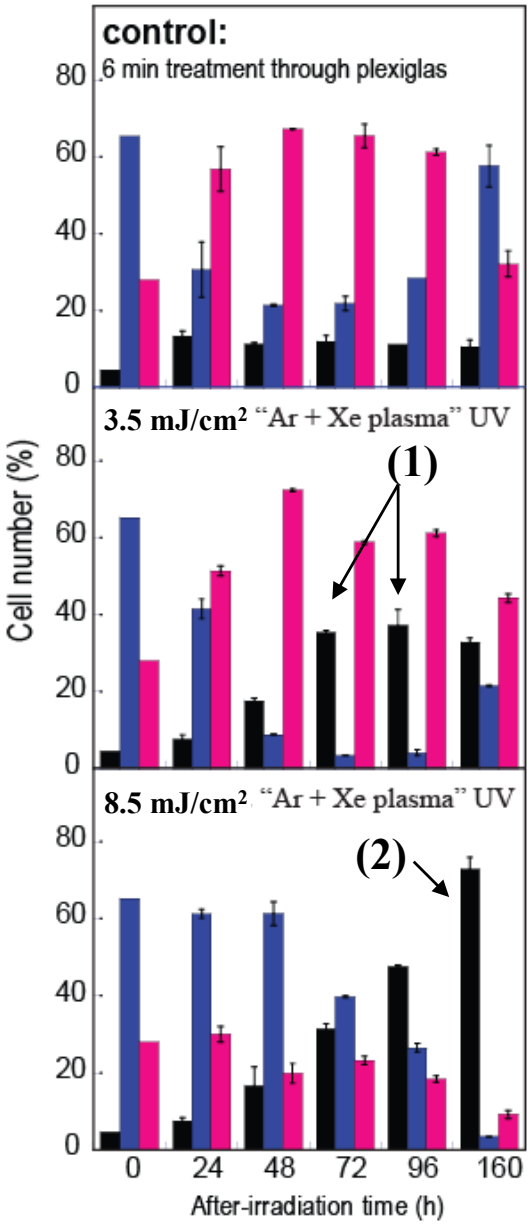
(2) Induction of late apoptosis (20-30%) 72 - 96 h after the treatment

~ 0.8 mJ/cm² UV 200-260 nm

UV

UV Radiation Produced by Ar+Xe Plasma

Effects on Human Skin Fibroblasts



- apoptotoc cells
- cells in G1 phase
- cells in S/G2 phases

Reminder: Pro-apoptotic dose of the total UV produced by "Ar plasma" = ***7.6 mJ/cm²*** *[0.8 mJ/cm² of short wavelength (200-260 nm) UV]*

(1) 3.5 mJ/cm² of total UV [1.0 mJ/cm² of short wavelength UV] produced by "Ar + Xe plasma" causes ~30% apoptosis 72 - 96 h after the treatment

(2) 8.5 mJ/cm² of total UV [2.6 mJ/cm² of short wavelength UV] produced by "Ar + Xe plasma" causes > 80 % dead cells

UV

UV Radiation Produced by Ar Plasma

Summary

- UV radiation is the major bactericidal factor of a short-term irradiation with Ar plasma.
- UV radiation generated by Ar plasma limits the duration of wound treatment (2 min/ treatment have been recommended for the clinical trial)
- The short-wavelength UV radiation (UVC) produced by plasma has the major damaging effect on human skin cell viability and proliferation. Cell response to UVC depends on intensity and spectrum.
- However, the pro-apoptotic dose of Ar plasma-generated UV is significantly higher than UV dose required for 100% bacterial density reduction in clear solutions

Effect (in PBS)	**Total UV *[UVC]* (mJ/cm^2)**	**Duration of plasma irradiation with MicroPlaSter (min)**
100% *E.coli* density reduction	**1.5** *[0.2]*	**0.8**
20-30% apoptosis in human skin fibroblasts	**7.6** *[0.8]*	**4**
Clinical study II.	**3.8** *[0.4]*	**2**

RS

Plasma-Generated Reactive Species

Methods

- Measurement of **total concentration of nitrates and nitrites** (NOx) in plasma irradiated liquids. This parameter reflects relative amounts of RS diffused from the gas phase during the irradiation with argon and air plasmas

- Measurement of Δ **pH** in liquids due to plasma irradiation

- Measurement of concentration of **hydrogen peroxide** (H_2O_2) in plasma irradiated liquids (with and without quartz glass). This parameter reflects the rate of liquid ionization by UV photons and ions

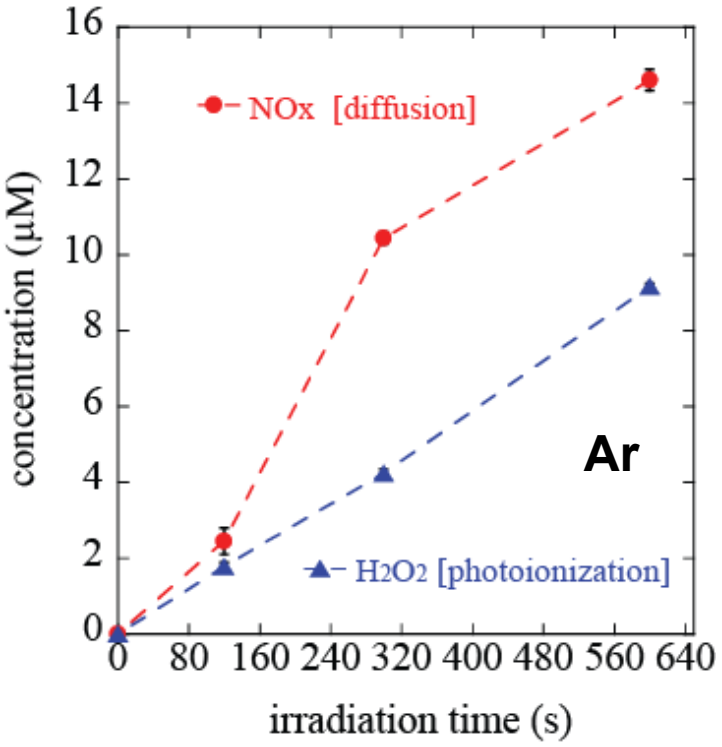
- Assessment of the effect of plasma-irradiated media on **bacteria** and **human cell** viability, migration and proliferation

RS

Plasma-Generated Reactive Species

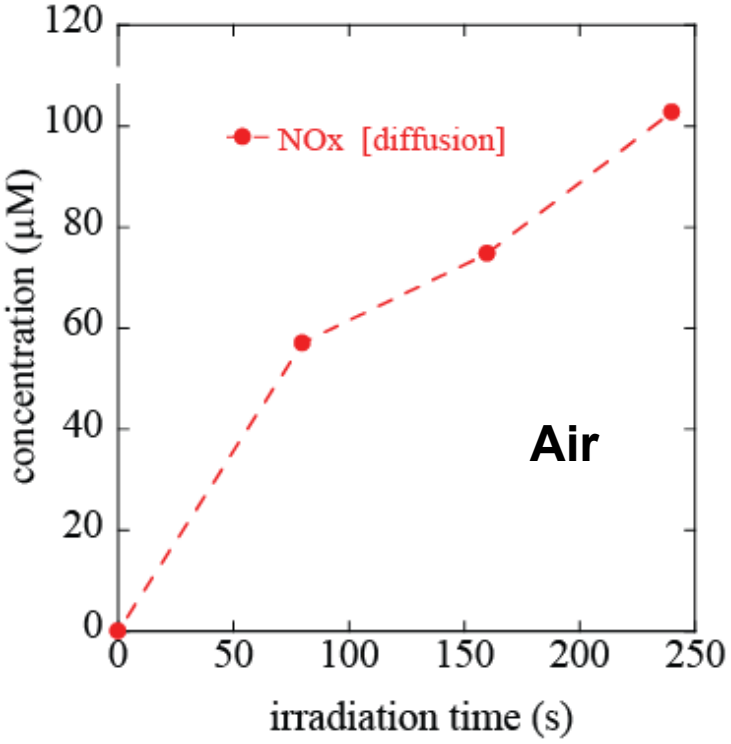
Plasma-irradiated liquids

Ar plasma (MiniPlaSter)



There are two main sources of RS in liquids irradiation with MicroPlaSter: diffusion from the gas phase and liquid photo ionization

Air plasma (FlatPlaSter)



No H_2O_2 have been detected

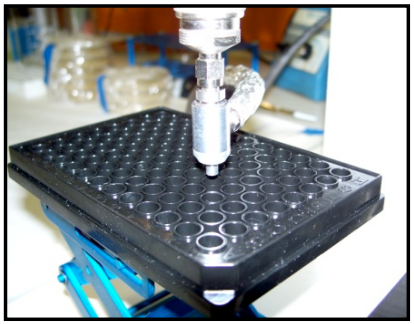
Diffusion from the gas is the only source of RS in liquids irradiation with FlatPlaSter

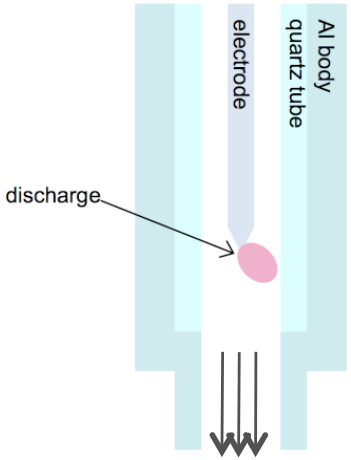
RS

Plasma-Generated Reactive Species

Liquid Ionization

MiniPlaSter - straight nozzle

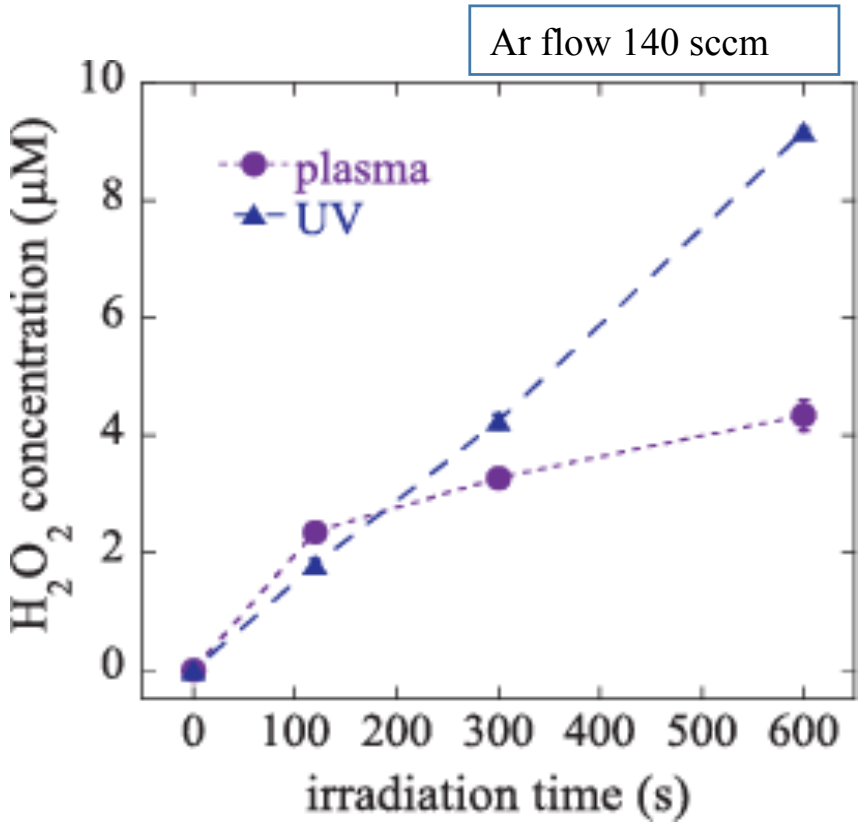




Charged particles
Reactive species
UV radiation

SAMPLE

Liquid ionization by Ar plasma-generated UV (MiniPlaSter - straight nozzle)

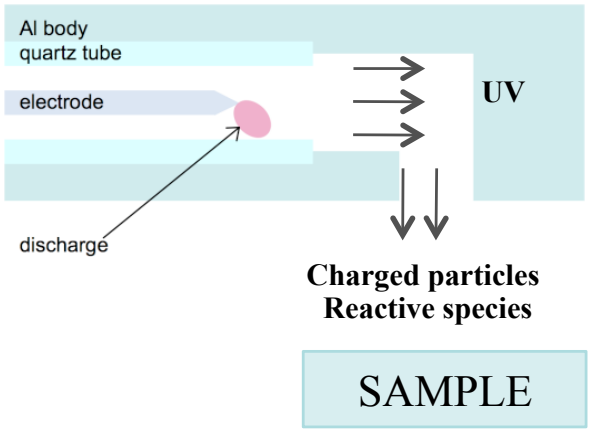


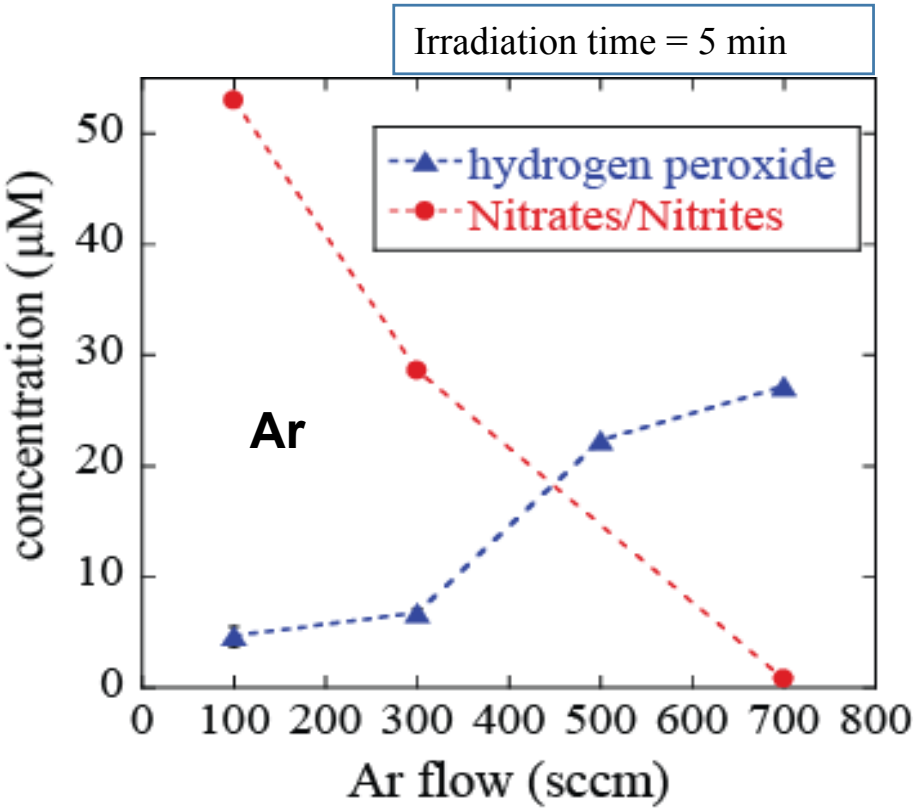
RS

Plasma-Generated Reactive Species

Liquid Ionization

Liquid ionization by Ar ions/excited–state atoms (MiniPlaSter with bent nozzle)



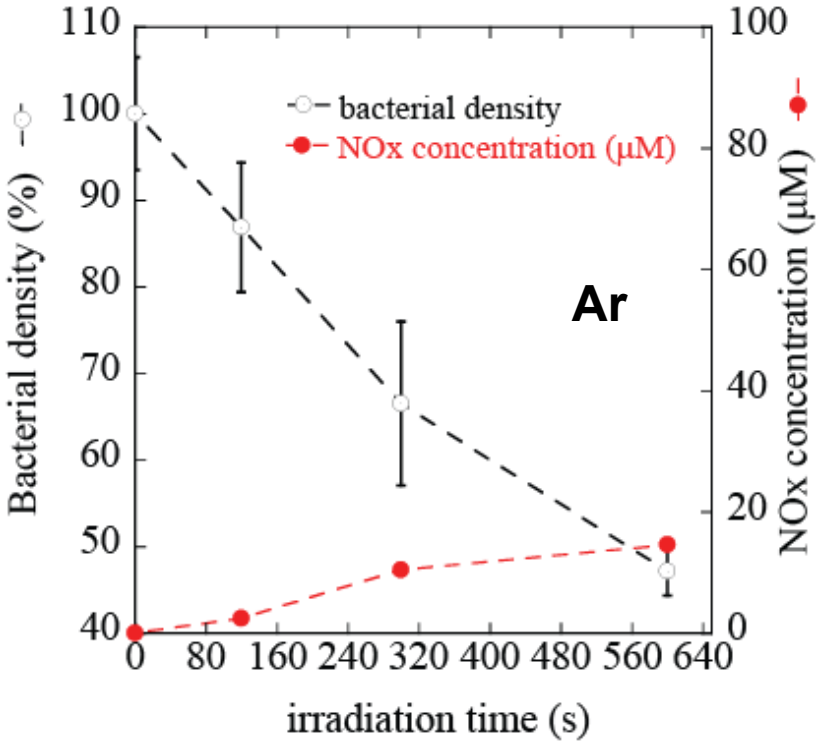


RS

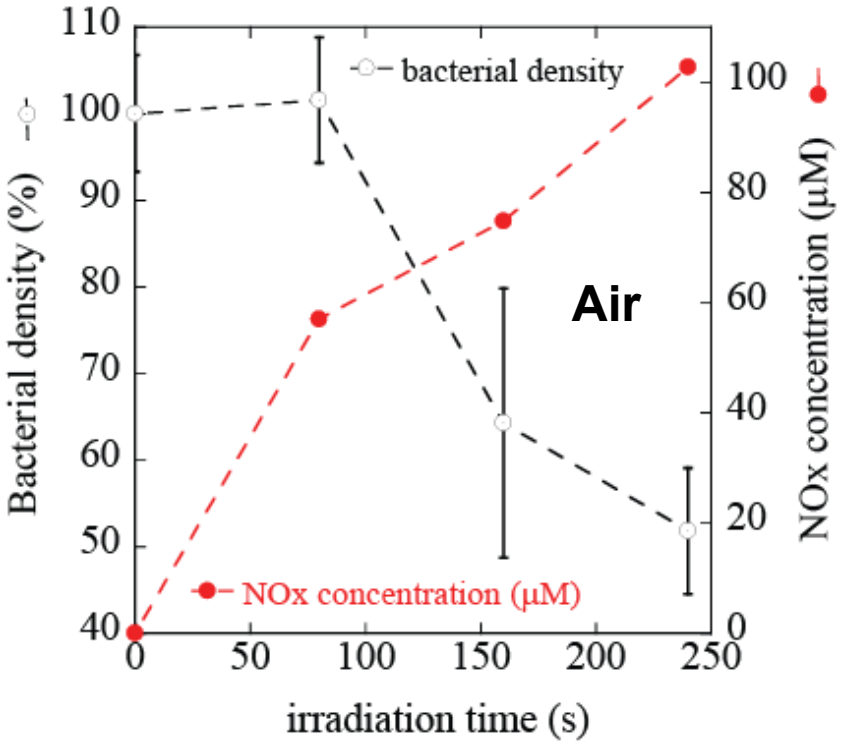
Plasma-Generated Reactive Species

Effect on Bacteria

Bacterial Density and Concentration of Reactive Nitrogen Species in Media Irradiated with Ar and Air Plasmas



E.coli was incubated for 1 hr in the LB medium irradiated with Ar plasma [no direct effect by UV!]



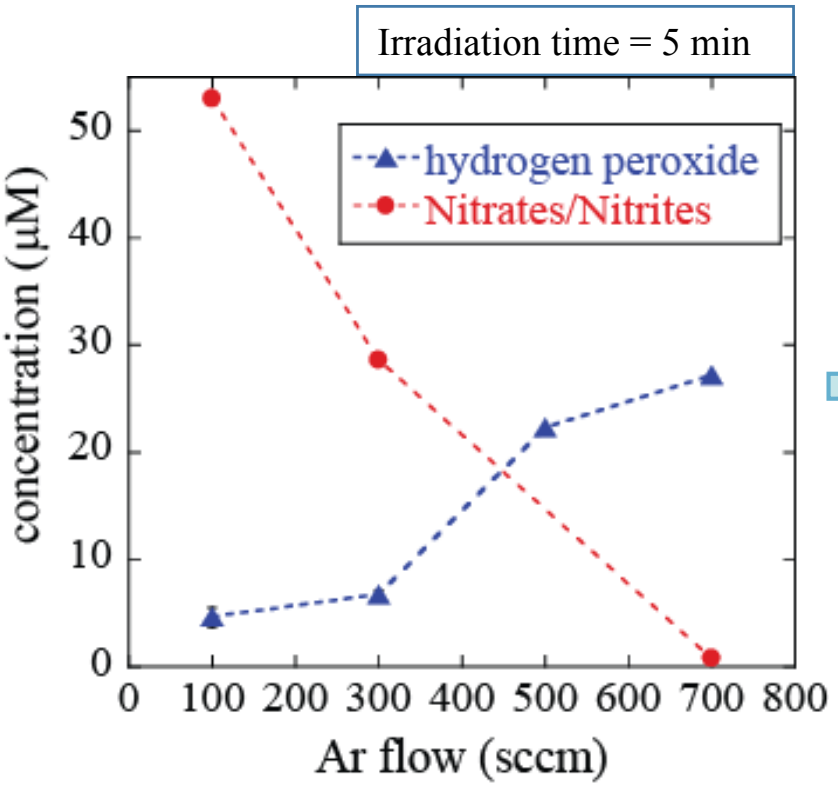
E.coli in LB medium was irradiated with Air plasma and incubated for 1 h

RS

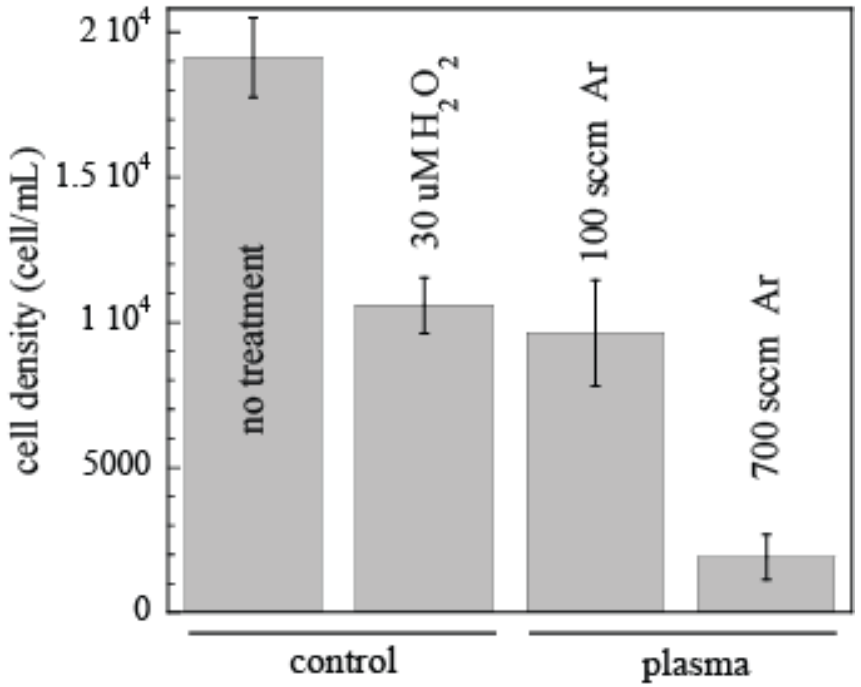
Plasma-Generated Reactive Species

Effect on Bacteria

Liquid ionization by Ar ions/excited–state atoms (MiniPlaSter with bent nozzle)



Bacterial density reduction due to irradiation with Ar plasma produced by MiniPlaSter-bent nozzle [no direct effect by UV!]



Reactive species produced by water ionization (H^+ and OH^-) have higher bactericidal effect than RS diffused from the gas phase and H_2O_2

RS

Plasma-Generated Reactive Species

Effect on Bacteria

- Effect of plasma irradiation on bacteria depends not only on the concentration of reactive oxygen and nitrogen species, but also on their composition

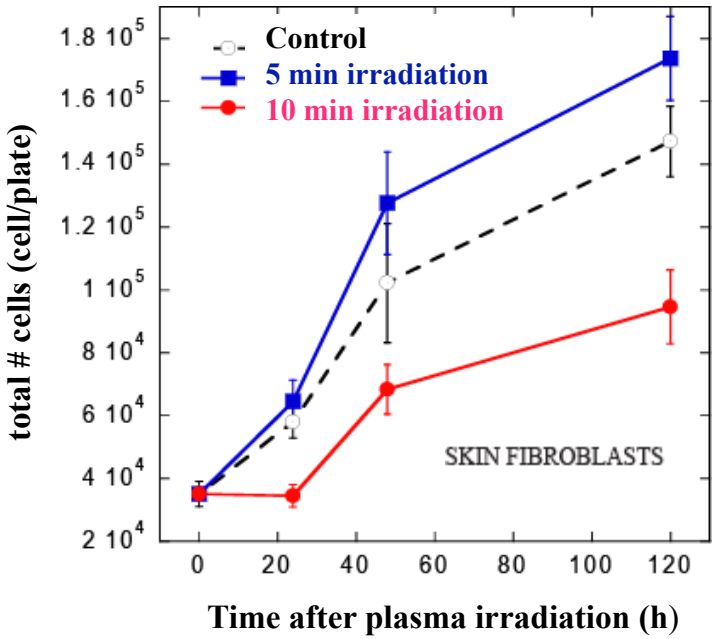
- Reactive species produced directly in liquids by plasma-generated UV radiation and bombardment with ion and excited-state molecules and atoms (OH, H, and H_2O_2) play important roles in liquid sterilization

RS

Plasma-Generated Reactive Species

Effects on Human Cells

Cell Proliferation in the Medium Irradiated with Argon Plasma (MicroPlaSter)

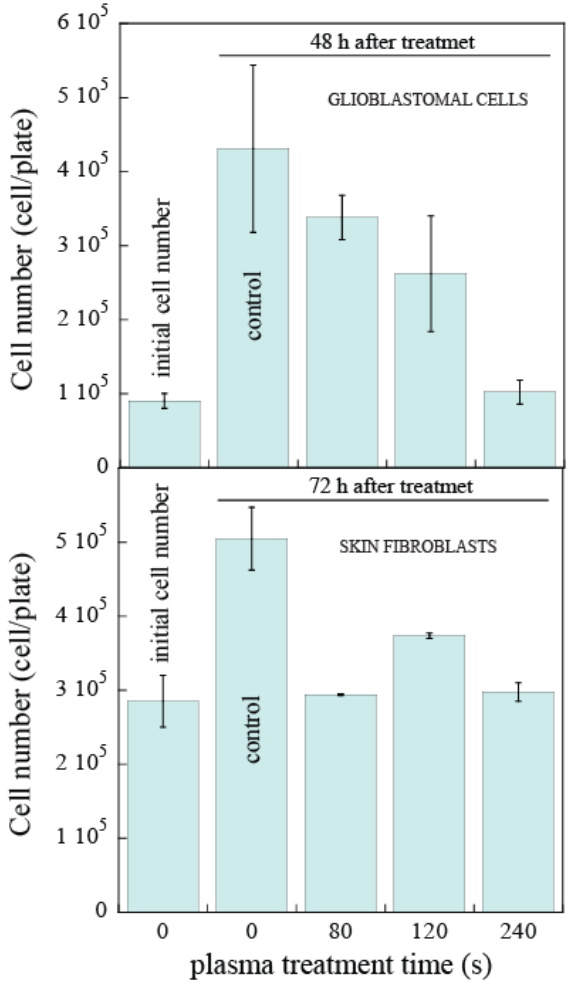


Ar

Incubation in the medium irradiated with argon plasma

- does not significantly affect fibroblast viability
- alters cell proliferation in a dosage-dependent manner: proliferation induction (5 min) or inhibition due to the temporary cell cycle arrest in G2 phase (10 min)

Inhibition of Cell Proliferation in the Medium Irradiated with Air Plasma (FlatPlaSter)



Air

Conclusions

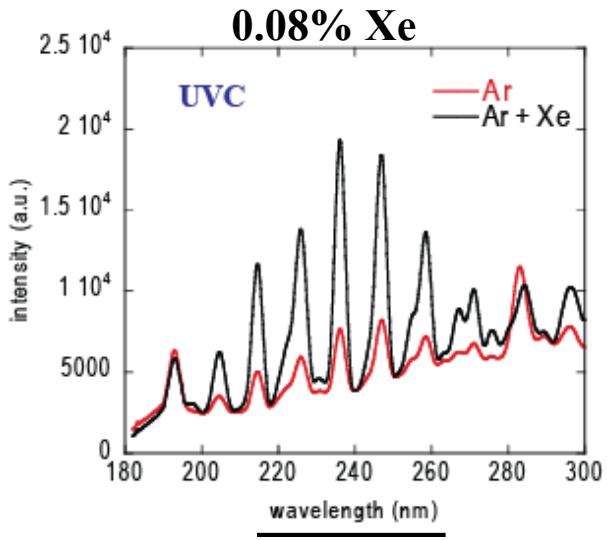
- Argon plasma has higher potential for chronic wound disinfection than air plasma.
- Plasma-generated UV radiation is important bactericidal component of argon plasma. It has direct and indirect (*via* reactive species) effects on biological systems.
- Significant bactericidal effect can be achieved even at low concentration of reactive species when combining several of them.
- Low concentrations of reactive species produced by argon plasma stimulate human skin cell proliferation .

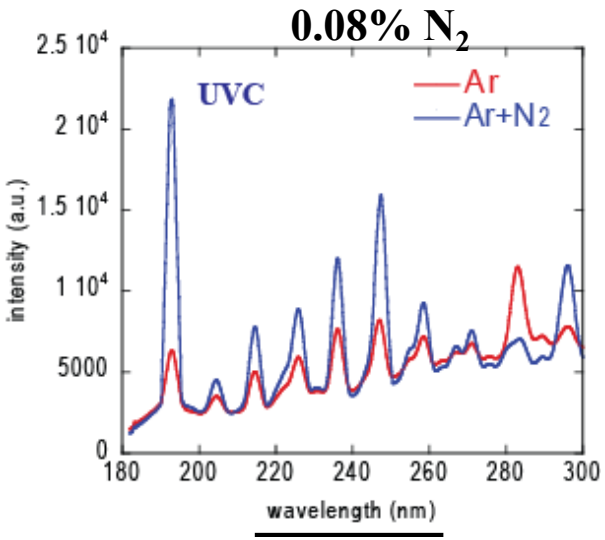
Problems

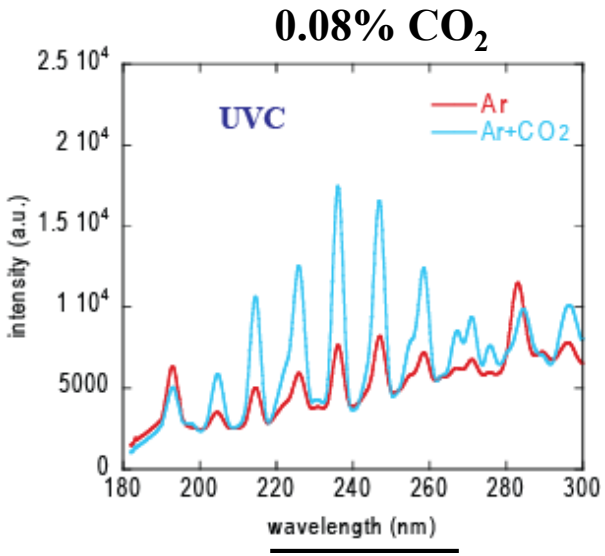
- UV radiation generated by MicroPlaSter (Ar plasma) limits the duration of plasma treatment of chronic wounds to 3-4 min
- Longer time (5-10 min) is required for accumulating reactive species produced by MicroPlaSter at concentrations toxic for bacteria
- The results of our study suggest that Argon plasmas for wound disinfection should be optimized in the following ways:
  - intensity of plasma-generated short wavelength UV (UVC) radiation on the object should be reduced
  - density of plasma-generated reactive species on the object should be increased in order to reduce the irradiation duration

UV

Modification of Ar plasma UVC spectra by Additives







UVC power	**6.7 µW/cm²** **7.5 µW/cm²**	**- // -** **12.0 µW/cm²**	**- // -** **12.9 µW/cm²**
wound treatment time limit:	**4 min** **3 min 30 s**	**- // -** **2 min 12 s**	**- // -** **1 min 56 s**

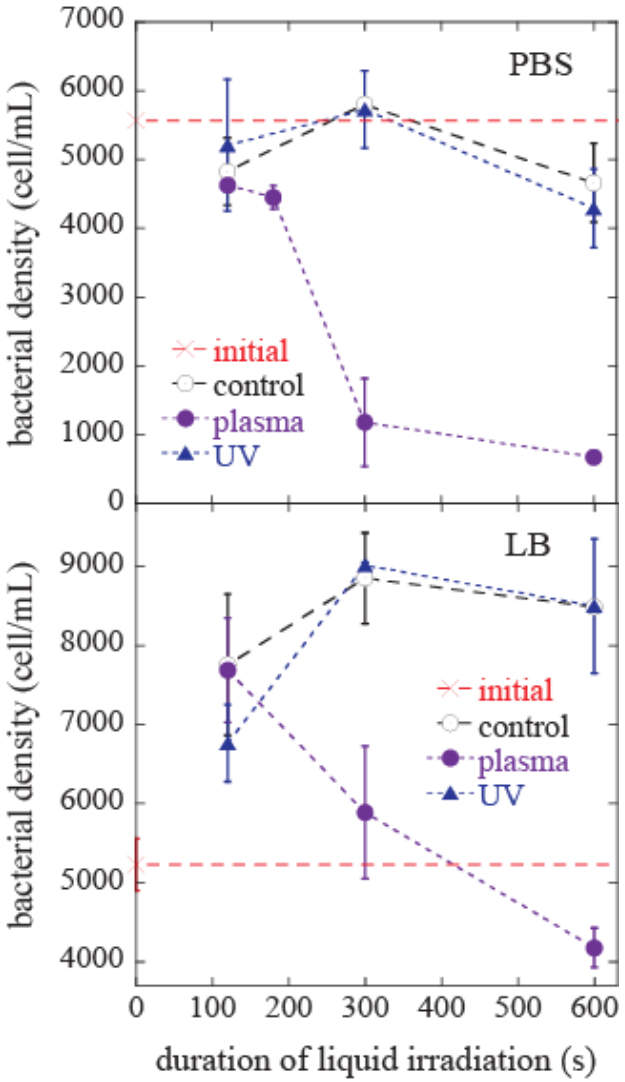
Questions


THANK YOU!

RS

Plasma-Generated Reactive Species

Effect on Bacteria



Argon plasma

- Ar plasma-irradiated liquids possess bactericidal and bacteriostatic properties
- The bactericidal and bacteriostatic properties of liquids irradiated with Ar plasma should be attributed to reactive species (*diffusion from the gas phase + liquid photoionization + liquid ionization by ions and excited state molecules and atoms*)

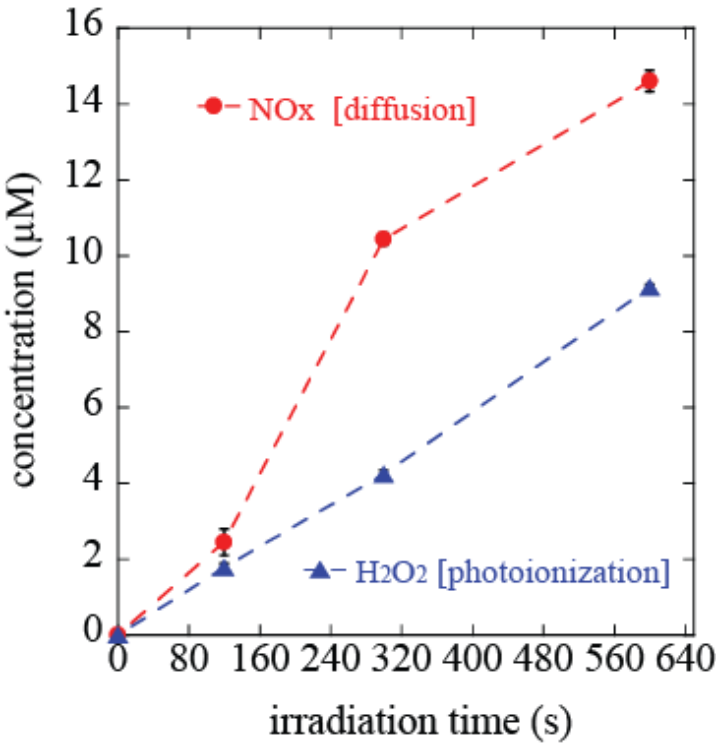
PBS – inorganic saline solution
LB – bacterial growth medium

RS

Plasma-Generated Reactive Species

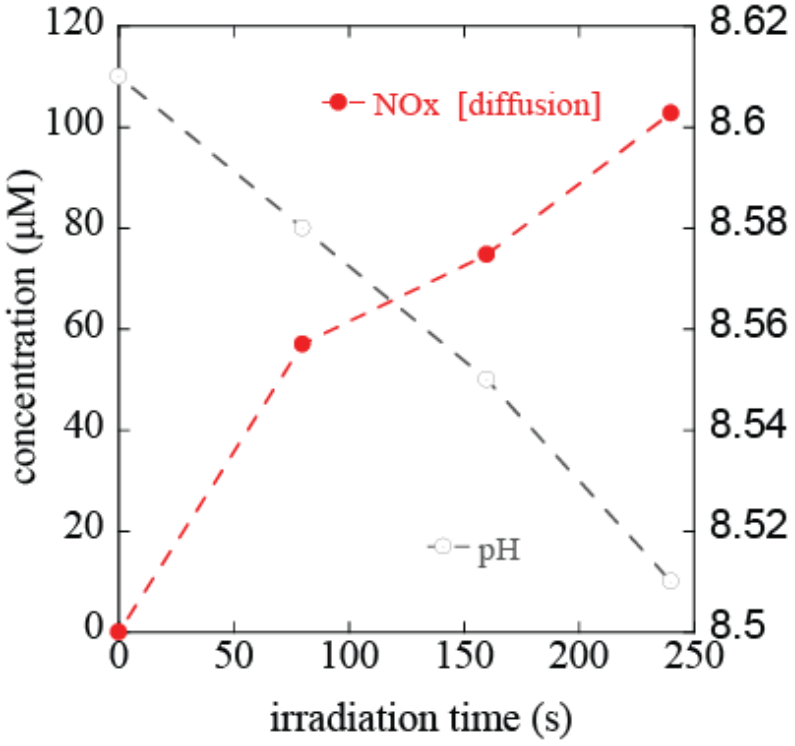
Plasma-irradiated liquids

Ar plasma (MicroPlaSter)



There are two main sources of RS in liquids irradiation with MicroPlaSter: diffusion from the gas phase and liquid photo ionization

Air plasma (FlatPlaSter)

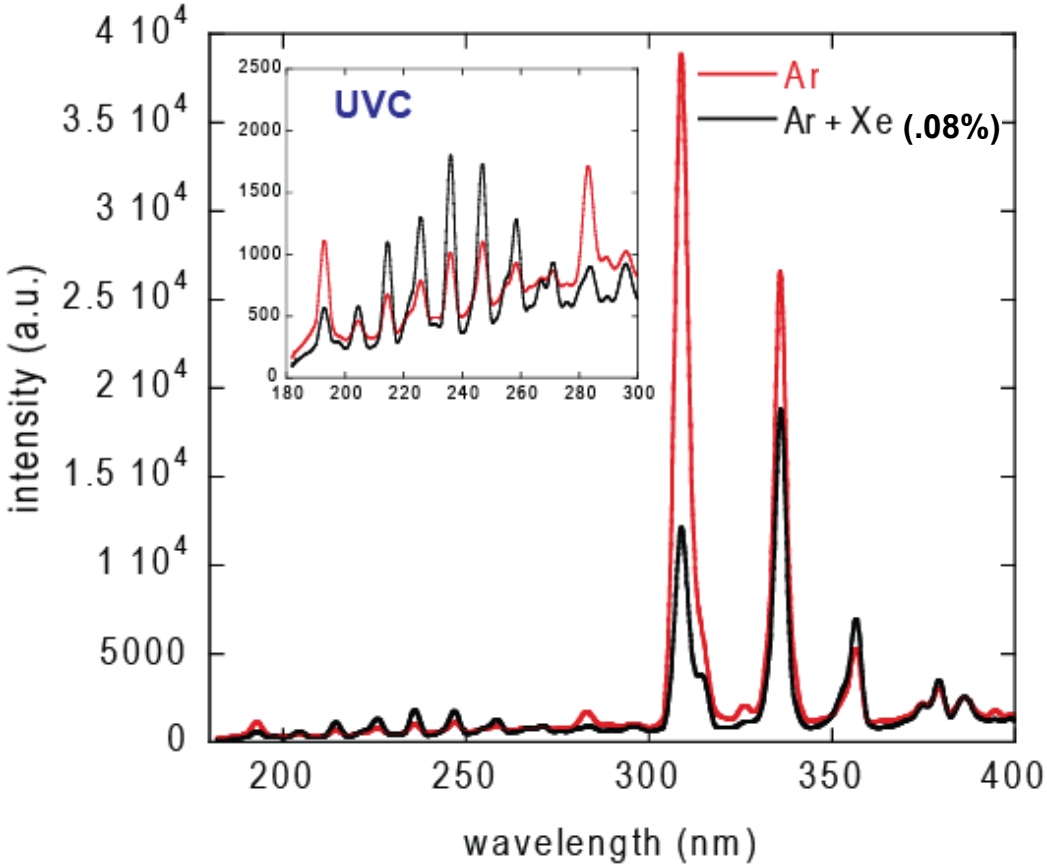


Diffusion from the gas is the only source of RS in liquids irradiation with FlatPlaSter

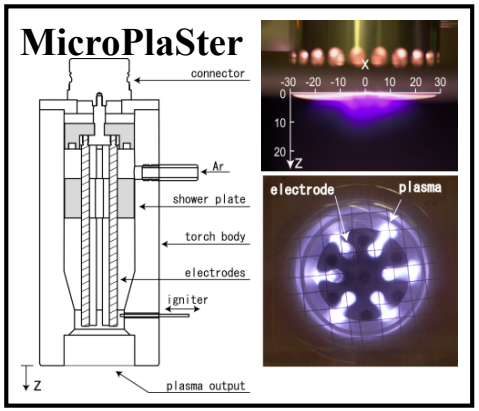
UV

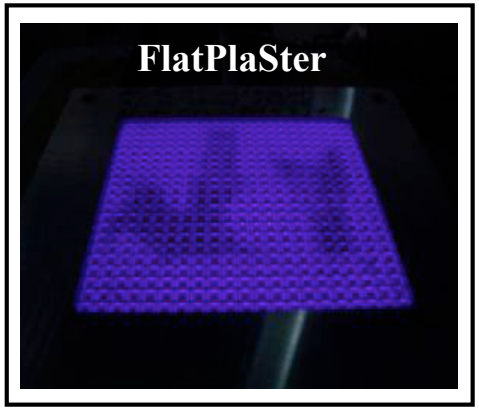
UV Radiation Produced by Ar Plasma

Effects on Human Skin Fibroblasts



Biologically Active Components of Plasmas (overview)





	Plasma factors and their effects on cells/ bacteria	Argon plasma *Power 60 W ; 1.2 sccm Ar flow; distance from the torch 20 mm*	Air plasma *9 kV; no gas flow; distance from the torch 20 mm*
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RS →	Liquid photoionization	~ 10 μM H_2O_2 (10 min irradiation)	no detectalbe H_2O_2 (10 min irradiation)
RS →	ROS and RNS diffusion from the gas phase	8 μM NO_2^-/NO_3^- (10 min irradiation)	> 300 μM NO_2^-/NO_3^- (10 min irradiation)
RS →	Liquid ionization by ions and excited molecules and atoms	Measured only for MiniPlaSter	no detectalbe H_2O_2 (10 min irradiation)

RS

Plasma-Generated Reactive Species

Effects on Human Cells

Composition of plasma-generated reactive species plays crucial roles in regulation of human cell proliferation