Osmotic pressure on cell membranes in a saline interacting with weakly ionized plasma

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LTP Seminar (Remote)

February 23 2021

Introduction

For disinfection, sterilization, cell cultures treatment (for example, enhancement of seed germination), different plasma sources are used. Almost everything that is generated in the plasma at the air-liquid interface and enters the physiological solution (RNS, ROS, O3, UV, etc) can have a physiological effect on cells.

A natural question arises: is plasma generated at air-liquid interface or inside a liquid a purely specific way of influencing cellular structures or is there something in common with well-studied and widely used cell biology and medicine?

We argue that osmosis is such a general phenomenon with physiological effects. It is surprising that this phenomenon and its role in plasma medicine was not discussed until very recently, until 2017. Moreover, taking account of osmosis makes it possible to predict changes in the shape of cells and their volume.

In today's seminar we will talk about this phenomenon and its manifestations at the plasma interaction with cellular structures in saline.

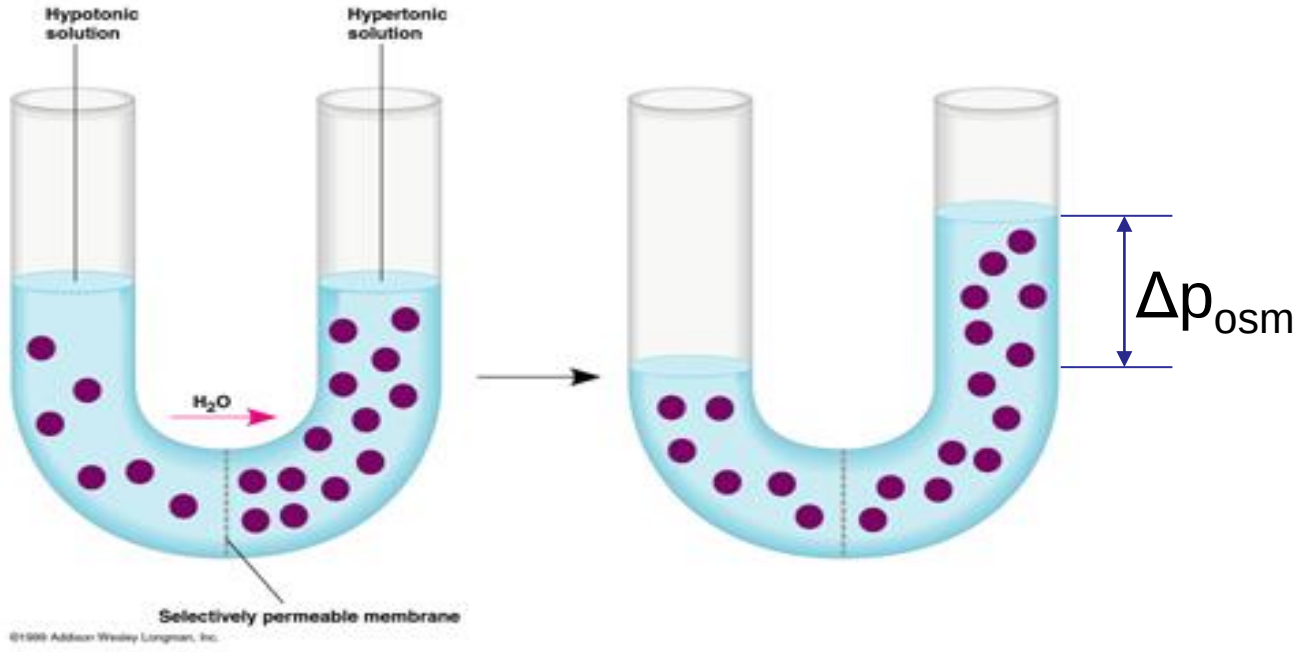
Outline

- Osmosis
- Water: ion composition, pH, etc…
- Solutions: Hypotonic, Hypertonic and Isotonic
- Experimental evidences
- Living cells in Petri dish interacting with plasma
- Selectivity
- Coronoviruses in a drop of aerosol
- Conclusions

Osmosis: the diffusion of water through a selectively permeable membrane.

Osmosis is the process in which water flows from an area with a low solute concentration, to an adjacent area with a higher solute concentration until equilibrium between the two areas is reached

- Passive transport
- Water molecules move from a higher concentration of water to a lower concentration of water
- Water will move to where there is a greater amount of solute (macromolecules, clusters and/or ions) because there is less water there.
- This additional water increases pressure and water stops flowing once the osmotic pressure is reached.



Δp_{osm} – osmotic pressure

van 't Hoff, 1855

$$\Delta p_{i,osm} = k_B T \, (n_{i,in} - n_{i,out})$$

$$\Delta p_{osm} = \Sigma(\Delta p_{i,osm})$$

https://estebanwhitmore.wordpress.com/2010/07/27/learning-by-osmosis/

Osmosis discovered in 1748 by Jean-Antoine Nollet (1700-1770)

The importance of osmosis

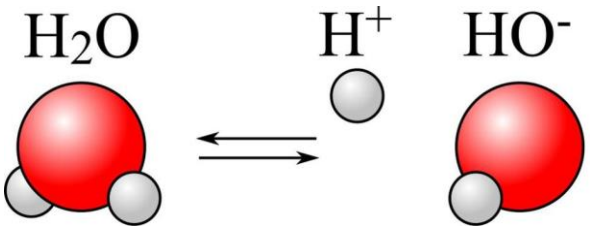
- ✓ Osmosis is responsible for the transport of nutrients in the trunks of tall trees, where capillary transport is unable to perform this function
- ✓ Elasticity, turgor of plant cells
- ✓ Elasticity of tissues, shape of organs
- ✓ Digestion
- ✓ The action of drugs
- ✓ Due to osmosis, water in the body is distributed between blood, tissues, cells
- ✓ Osmosis plays an important role in many biological processes at the cellular and tissue levels
- ✓ Plasma medicine?

Reverse osmosis is a process in which, at a certain pressure, a solvent (usually water) passes through a semi-permeable membrane from a more concentrated to a less concentrated solution, that is, in the opposite direction for osmosis. Reverse osmosis has been used since the 1970s for water purification, for obtaining drinking water from seawater, for obtaining highly pure water for medicine, industry and other needs.

Electro-osmosis is the movement of a liquid through capillaries or porous membranes when an external electric field is applied.

Water: ion composition, pH, etc…

In pure water, the concentrations of hydrogen ions [H^+] and hydroxide ions [OH^-] are the same and at 22° C they are 10^{-7} mol/liter



$$N_{H^+} = N_{OH^-} = 10^{19}\ \mathrm{m}^{-3}$$

D. Eizenberg, W. Kauzmann, The structure and properties of water (1969)

$$H_2O \rightarrow OH^- + H^+$$
$$H^+ + H_2O \rightarrow H_3O^+$$
$$H_3O^+ + OH^- \rightarrow 2H_2O$$

https://en.wikipedia.org/wiki/Hydronium

For convenience of presentation, in order to get rid of the negative exponent, instead of the concentration of hydrogen ions, it is used the decimal logarithm taken with the opposite sign, which, in fact, is the hydrogen exponent - pH.

$$\mathrm{pH} = -\lg[\mathrm{H}^+]$$

pH=7 - neutral solutions

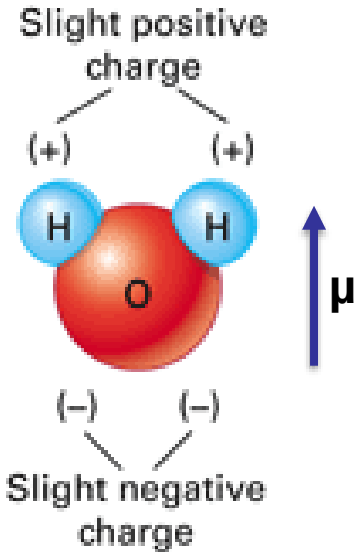
pH<7 - acidic solutions

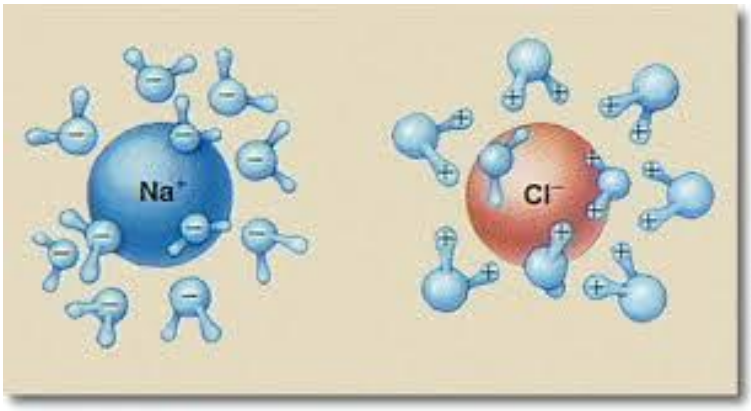
pH >7 basic solutions

With decreasing temperature, the neutral pH increases.

Hydrated (solvated) ions

Water molecule H_2O – polar molecule with permanent dipole moment μ=1.84 D





Hydrated Sodium (cations) and Chlorine (anions) ions in water

What is the difference between dissolved neutral molecules and ions in water?

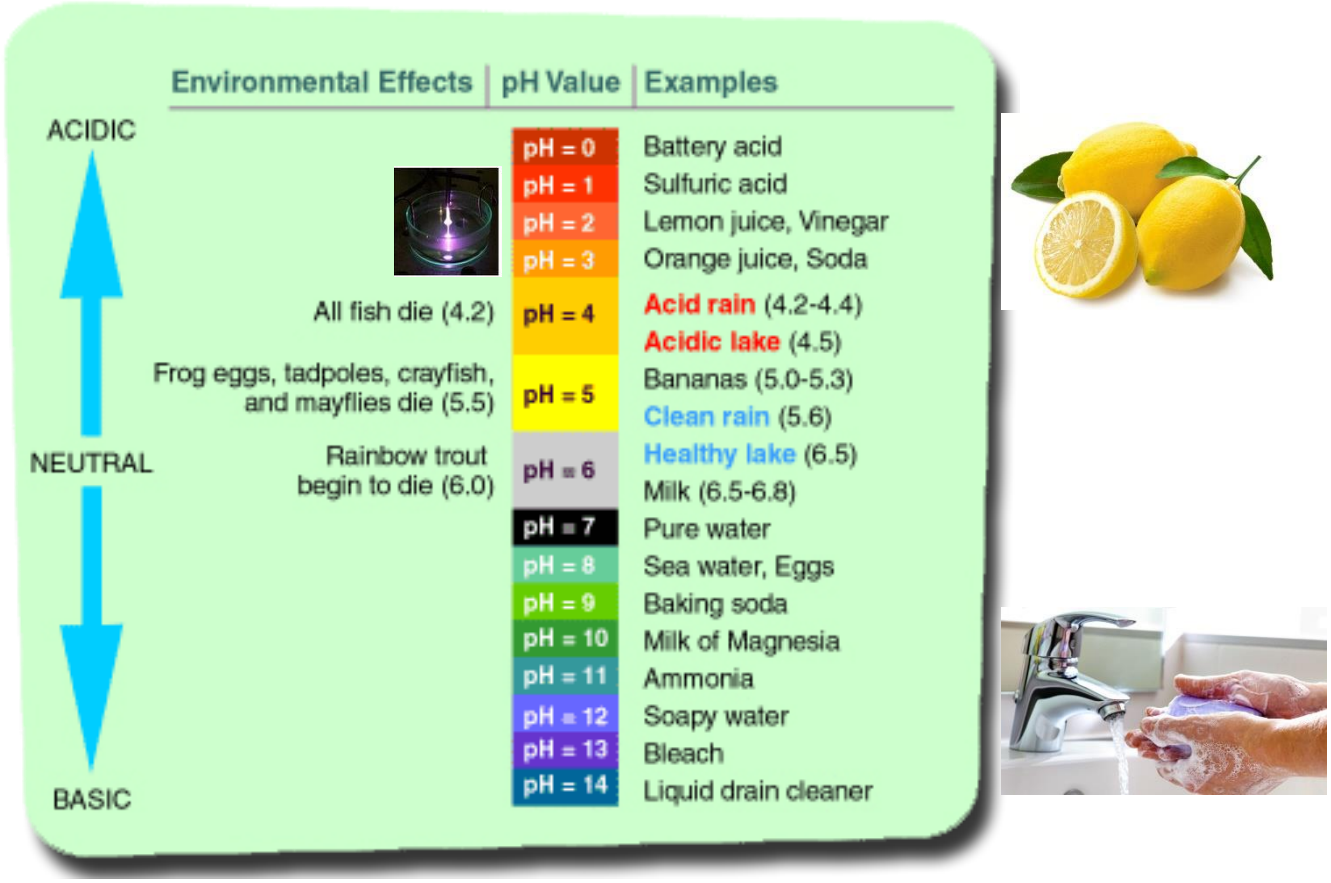
Solvation (from Lat. *Solvo* "dissolve") - electrostatic interaction between particles (ions, molecules) of a dissolved substance and a solvent. **Solvation in aqueous solutions** is called **hydration**.

The ions become hydrated, i.e. acquire a shell of polar water molecules. This significantly increases their effective size and weight. This leads to a significant decrease in the mobility and diffusion coefficient, a significant slowdown in recombination, etc.

One of the main effects on the physiological effects of plasma is the impermeability of the lipid membrane for hydrated ions formed from those coming from the plasma.

As we will see below, plasma treatment of water (saline) increases its acidity.

It is well known that acidic solutions (low pH, lemon juice, for example) and alkaline solutions (high pH, soap solution, for example) are excellent disinfectants that kill viruses and pathogenic bacteria.



https://www.usgs.gov/media/images/ph-scale

Blood has a normal pH range of 7.35 to 7.45. This determines the normal osmotic pressure in viruses and cells.

Low and high pH alter the osmotic pressure in viruses and bacteria and makes them unviable.

An example of air plasma-water interface

Air

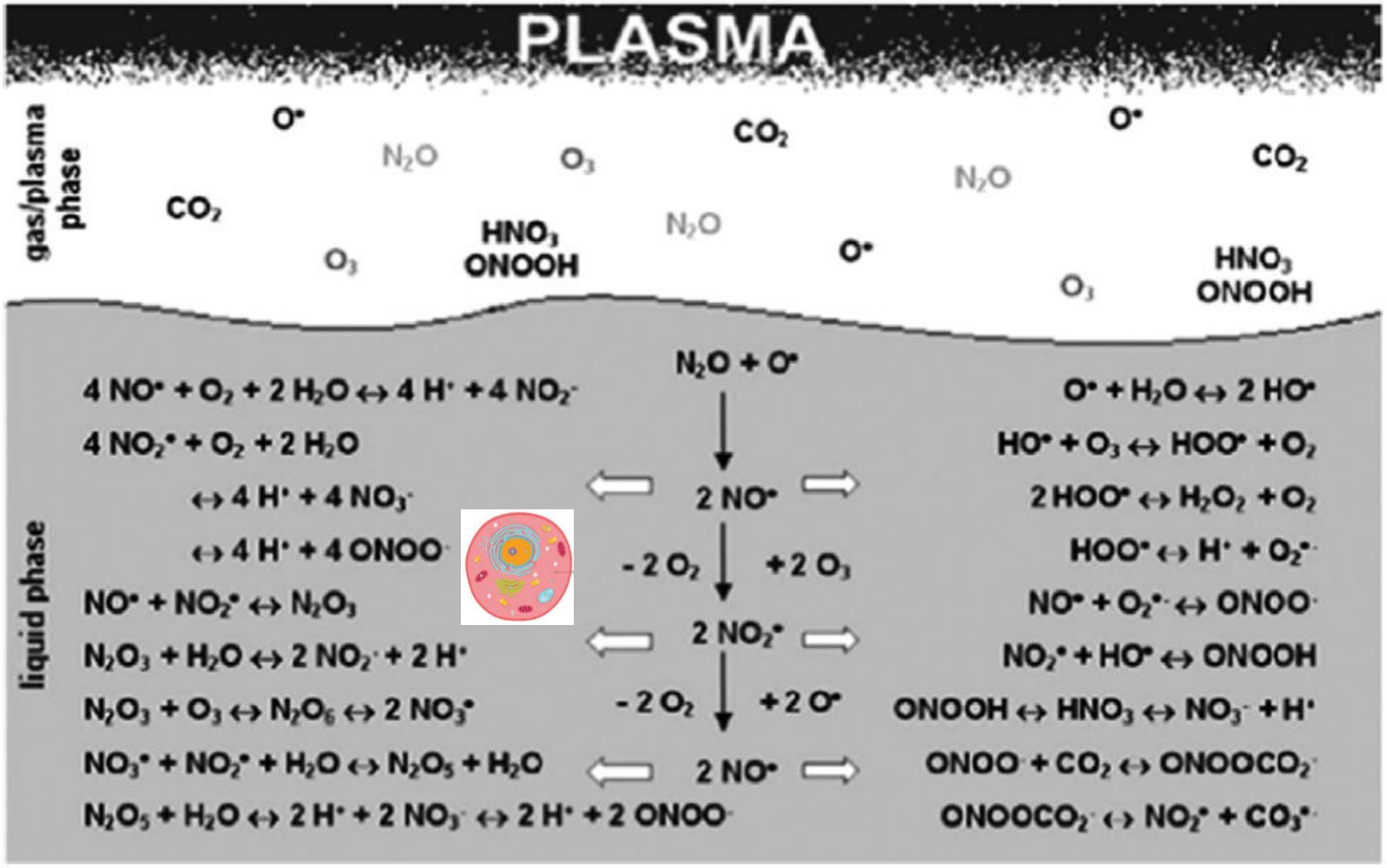
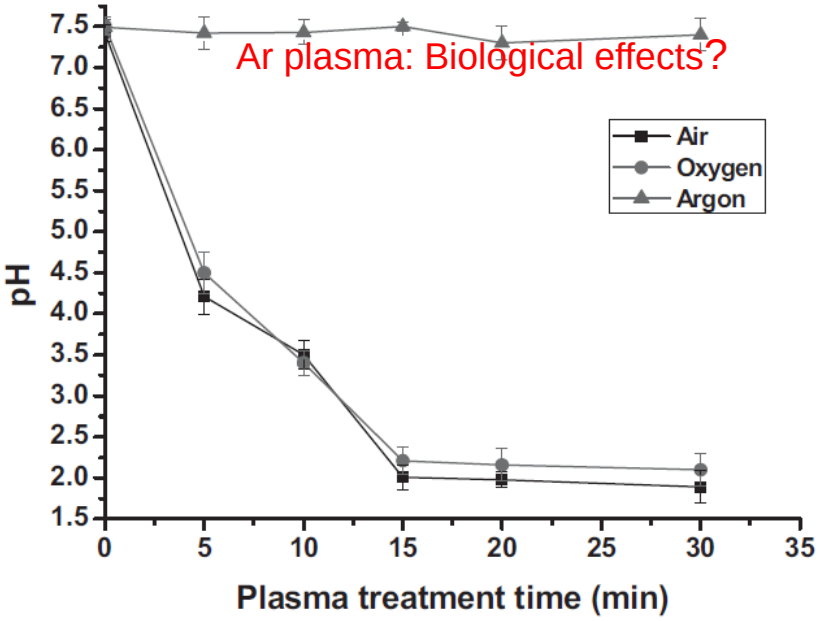


Fig. 1. Assumption of chemical reactions in plasma treated liquids [54] ©2011 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

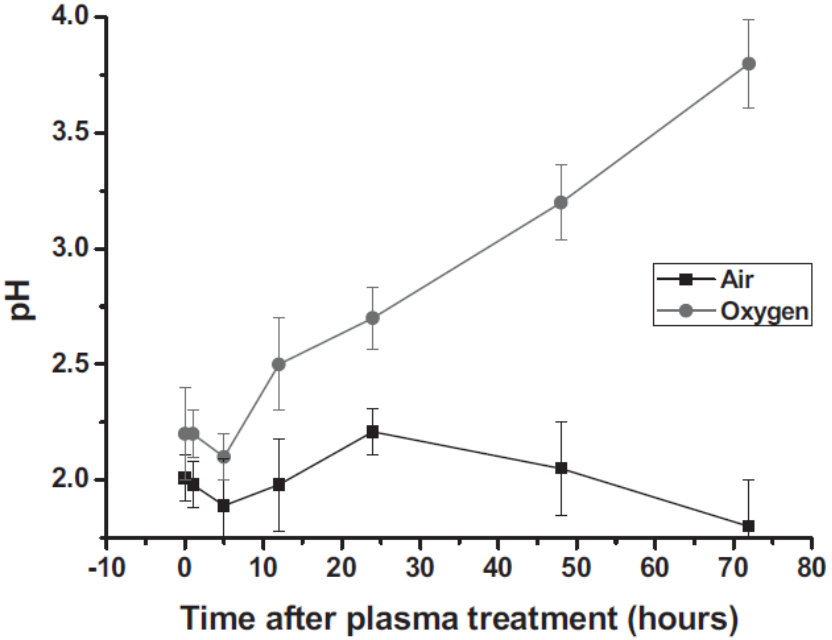
pH in plasma and plasma activated water (PAW)

Changing in ion composition may result in acidity of plasma treated water. Many papers reported measurements of pH in water interacting with plasma since 1998: B. Benstaali et al, Eur. Phys. J. AP 4, 171–179 (1998)

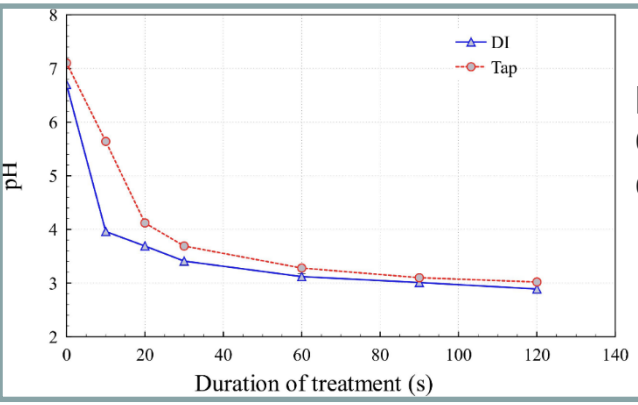
N. Shainsky, D. Dobrynin, …, G. Fridman, Y. Cho, A. Fridman, G. Friedman, Plasma Acid: Water Treated by Dielectric Barrier Discharge, Plasma Process. Polym. 2012



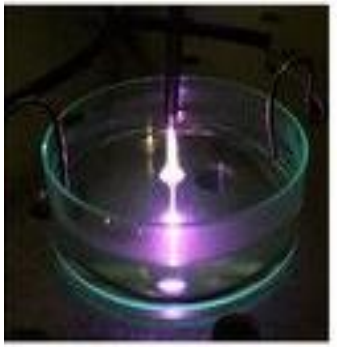
Variations of pH of deionized water after plasma treatment in three different gases: air, oxygen and argon



Results of pH changes of deionized water as a function of time after plasma treatment.



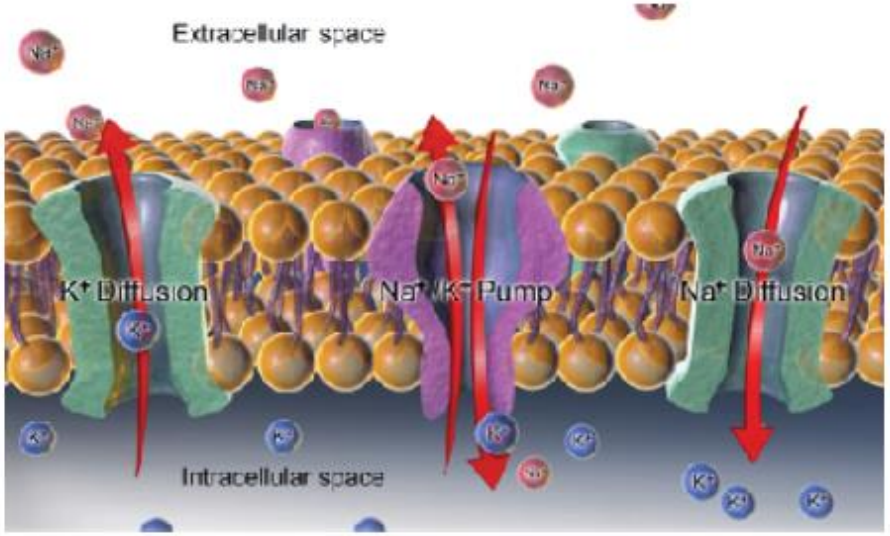
pH value of DI and tap water as a function of the duration of plasma treatment (non-equilibrium low current arc in air)



V. Gamaleev et al, Appl. Sci. 2019, 9, 3505

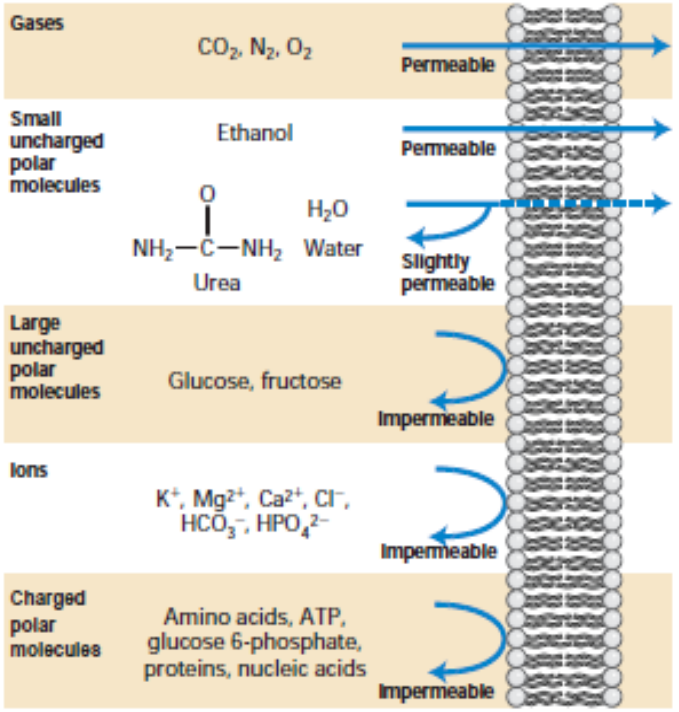
Osmosis and transport through biological membranes

- Water diffuses almost freely through biological lipid membranes
- Solute (***solvated, hydrated ions and molecular clusters***) are too large to move across
- Cell membrane is impermeable for charged particles
- Cells use voltage-gated ion channels and ion pumps to regulate concentration of ions outside and inside the cell volume



$p_{in}=p_{out}+2\sigma/R+\Delta p_{osm}$

Δp_{osm} controllable by plasma



▲ FIGURE 7-1 Relative permeability of a pure phospholipid bilayer to various molecules. A bilayer is permeable to small hydrophobic molecules and small uncharged polar molecules, slightly permeable to water and urea, and essentially impermeable to ions and to large polar molecules.

H. Lodish, et al, Molecular Cell Biology (W. H. Freeman, 2008) Ch.7

Osmotic pressure depends only on temperature and concentration difference across the membrane!

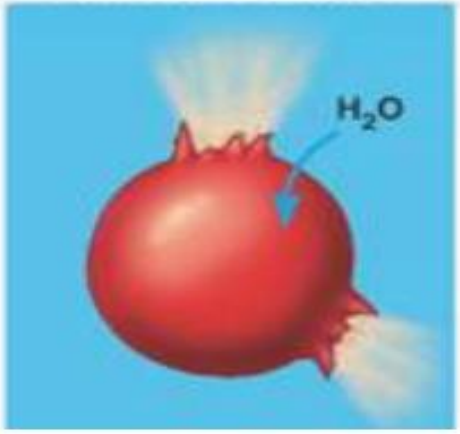
$\Delta p_{i,osm}=k_B T\,(n_{i,in} - n_{i,out})$; $\Delta p_{osm}=\Sigma(\Delta p_{i,osm})$ (van 't Hoff, 1855)

Solutions

Hypotonic Solution - external solution has a lower concentration of solute than internal

Isotonic Solution - both solutions have same concentrations of solute

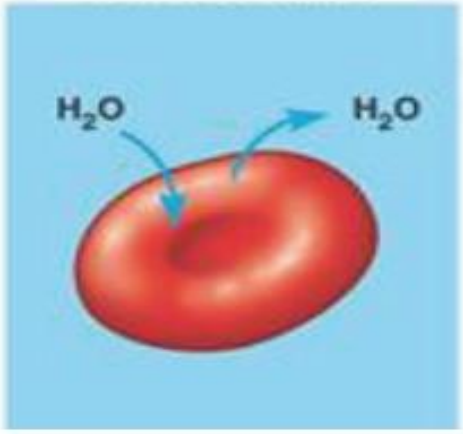
Hypertonic Solution - external solution has a higher concentration of solute than internal



hypotonic solution

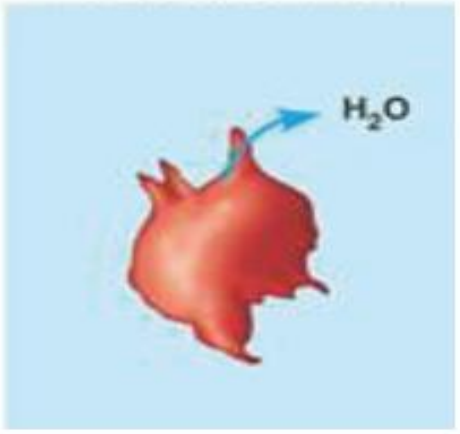
$n_{i,in} >> n_{i,out}$

Cell death - *Necrosis: loss of cell membrane integrity; leakage of cellular content*



isotonic solution

$n_{i,in} \sim n_{i,out}$

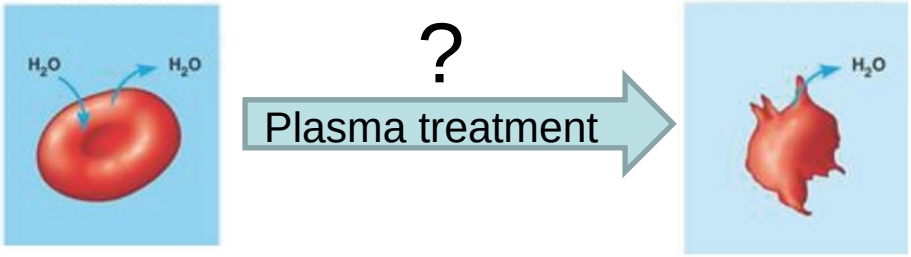


hypertonic solution

$n_{i,in} << n_{i,out}$

Cell death - *Apoptosis*

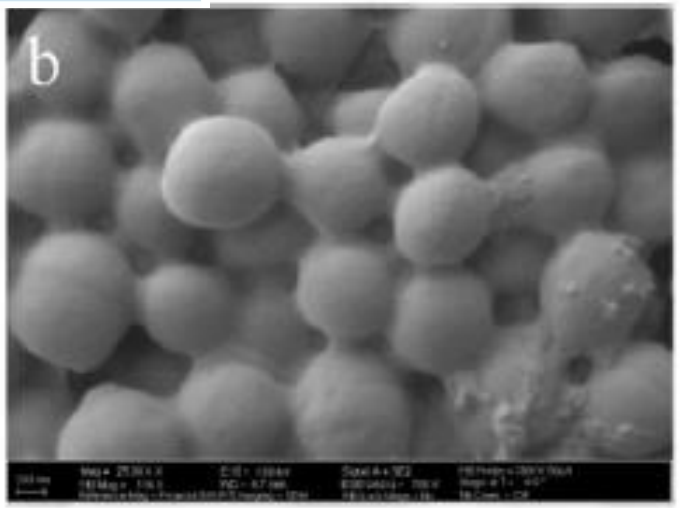
We predicted (2018) the most probable **“hypertonic scenario”**



M.N. Shneider, M. Pekker, J.Appl.Phys. 123, 204701 (2018)

Evidence

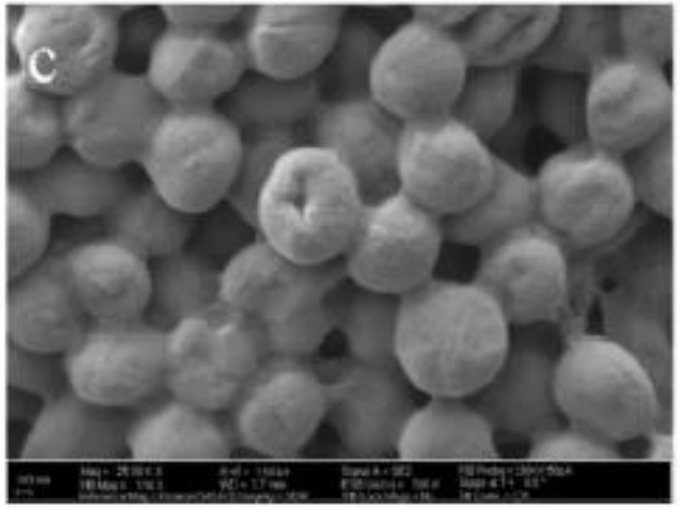
aureus biofilms



b) untreated water

PAWs killed bacteria by inducing cell membrane damage

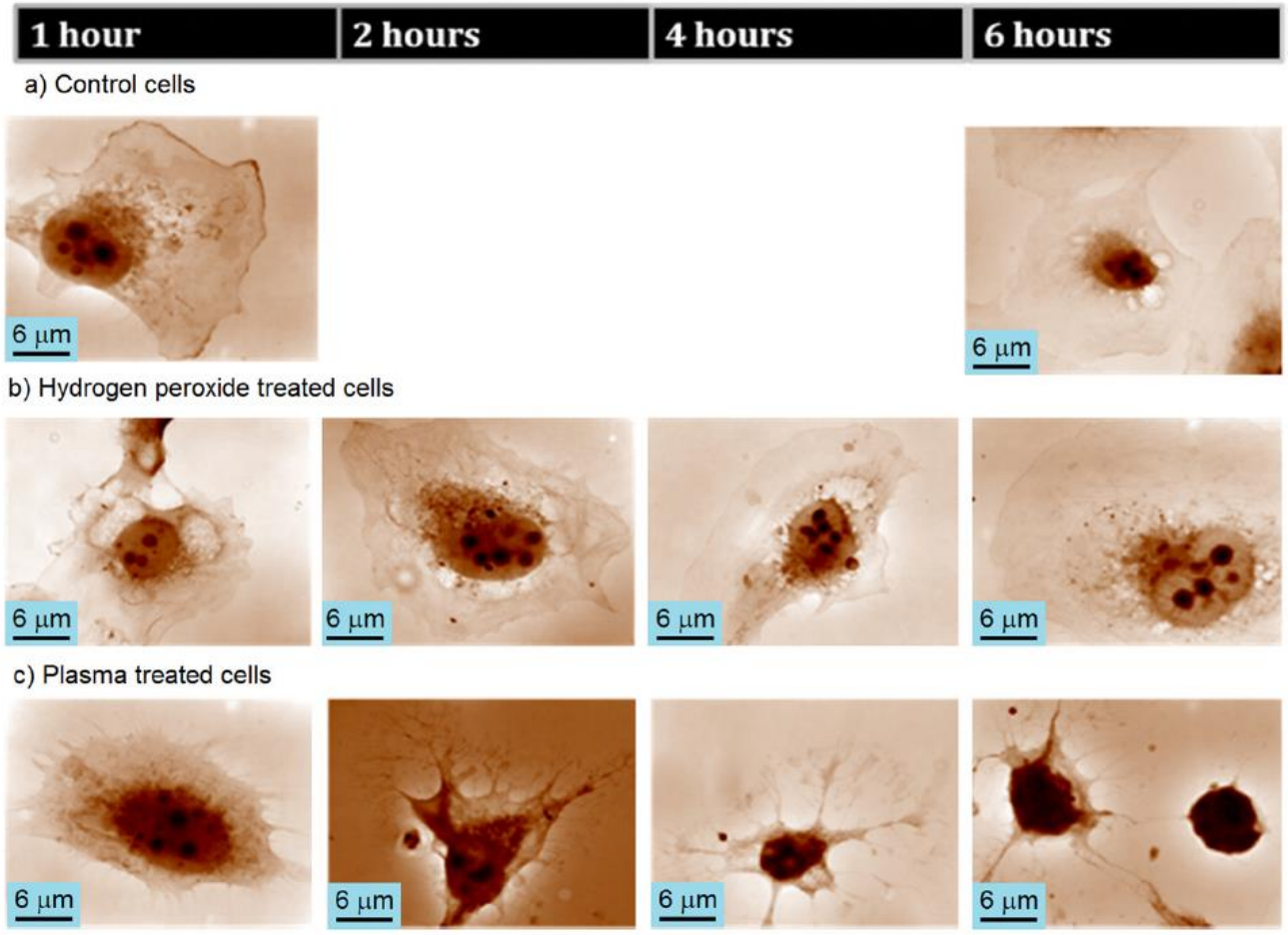
The morphology of bacteria in the biofilm without treatment (Fig. 2b) shows integrity and has smooth surface. However, PAW disrupted bacterial cell integrity, formed pits, and caused shrinking and distortion on the cell wall (Fig. 2c).



c) treated with PAW-Air-30 (30 min activated with plasma) for 3 hour

Tung-Po Chen et al., Environ Sci Pollut Res (2018)

Examples of hypotonic and hypertonic cell deformation



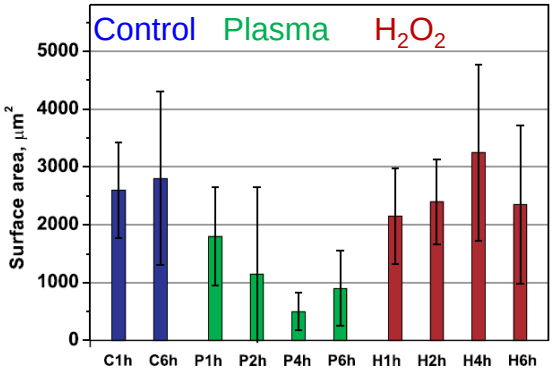
The human thyroid epithelial cells in physiological solution in Petri dish

H_2O_2

Hypotonic solution

DBD plasma in air-liquid gap. 9 kV nanosecond pulses : 10000 pulses, 300 Hz

Hypertonic solution



APPLIED PHYSICS LETTERS 106, 233703 (2015)

Phase imaging microscopy for the diagnostics of plasma-cell interaction

Yolanda Ohene,[1,a)] Ilya Marinov,[1,3,a)] Lucie de Laulanié,[2,a)] Corinne Dupuy,[3] Benoit Wattelier,[2] and Svetlana Starikovskaia[1]

Interaction with plasma

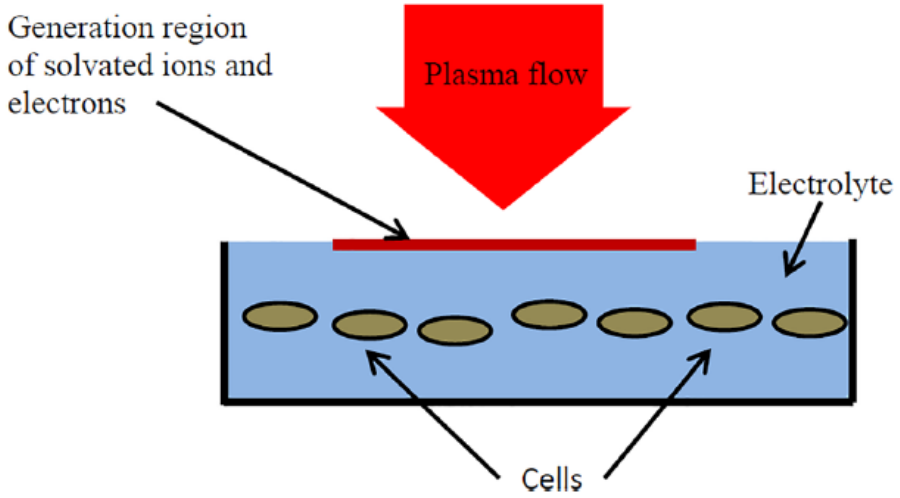


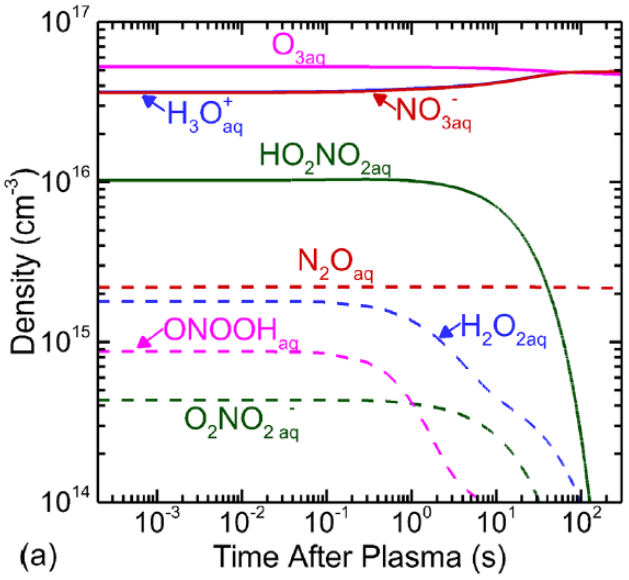
FIG. 1. Schematic of the experimental setup for a plasma jet acting on cells in a Petri dish.

The density of charged particles in a saline solution of electrolyte, corresponding to a living organism, is $n_{i,0} \approx 0.3$ M/L $\approx 2 \times 10^{26}$ m^{-3}

The characteristic Debye screening length, $\Lambda_D < 1$ nm

Additional solvated (hydrated) ions, electrons and molecular clusters are formed on the liquid-plasma boundary

The lifetime of solvated ions and neutral molecular clusters (RNS, ROS, etc) formed in the saline is long enough to neglect decay and treat them as stable.



An excellent example:

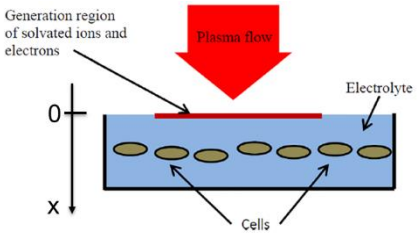
Liquid in DBD.

The decay of reactivity in liquid after the plasma treatment. Time is relative to the end of the last pulse.

Figure taken from: *Amanda M Lietz and Mark J Kushner 2016 J. Phys. D: Appl. Phys. 49 425204*

Another example: the lifetime of ions NO_3^-, NO_2^- and molecules H_2O_2 is of the order of several hours! [*P. Lukes et al., Plasma Sources Sci. Technol. 23, 015019 (2014)]*

Diffusion of solvated ions in a Petri dish



Approximations:

1. We neglect the processes of ion recombination in a physiological solution

2. The radius of the region of interaction of the plasma with the surface of the liquid is greater than the distance from the surface to the cells, so the problem of diffusion of solvated charged particles (and neutral molecules) can be described by a 1D diffusion equation

$$\frac{\partial n}{\partial t} = D\frac{\partial^2 n}{\partial x^2}$$

Boundary conditions:

in the injection region (surface) $D\frac{\partial n}{\partial x}\Big|_{0,t>0} = J\theta(t_0 - t), \quad \theta(t_0 - t) = \begin{cases} 1 \text{ at } t < t_0 \\ 0 \text{ at } t \geq t_0 \end{cases}$

and at “infinity” $n_\infty = 0$

Solution: $$\mu = \int_0^\tau \frac{1}{\sqrt{\pi(\tau - \zeta)}} \exp\left(-\frac{\xi^2}{4(\tau - \zeta)}\right)\theta(1 - \zeta)d\zeta$$

Dimensionless variables: $\tau = \frac{t}{t_0}, \quad \xi = \frac{x}{l_0} = \frac{x}{\sqrt{Dt_0}}, \quad \mu = \frac{n}{n_0} = \frac{n}{J}\sqrt{\frac{D}{t_0}}$

Typical values for the diffusion coefficient: $D \sim 10^{-9} - 5 \times 10^{-9}$ m^2/s

At t_0~100 s, l_0~$(Dt_0)^{1/2}$ ~ 1 mm

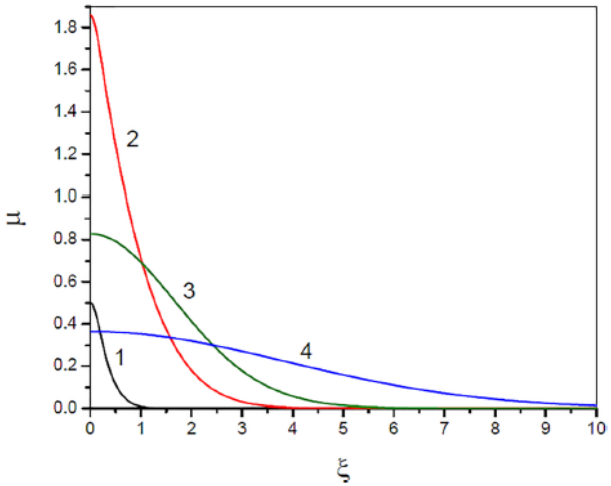


FIG. 3. Solution of the diffusion equation in dimensionless variables. Line 1 corresponds to $\tau = 0.1$, 2 to $\tau = 1$, 3 to $\tau = 2$, and 4 to $\tau = 4$.

Solvated ions have enough time to diffuse to the cells that are in the upper layers of physiological solution in a Petri dish (with a typical depth of several millimeters).

M.N. Shneider, M. Pekker, J.Appl.Phys. 123, 204701 (2018)

Estimate of the variation of the osmotic pressure on the cell membrane

Assuming, for simplicity, that the Laplace pressure $p_L = 2\sigma/R$ is small, the equilibrium radius of the cell is determined by the ion and molecular cluster densities inside and outside the cell.

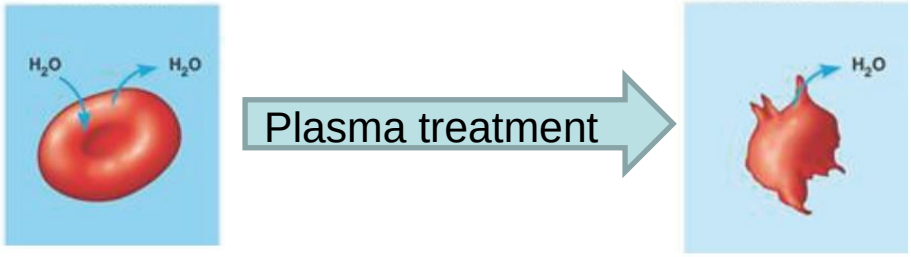
An additional contribution to the equilibrium osmotic pressure, as a result of the interaction of the fluid with the plasma, $\delta p_{osm} = k_B T \delta \sum n_i$.

As shown, for example, in [P.Lukes et al, PSST, 23, 015019 (2014)]: the densities of ions NO_3^- , NO_2^- , and molecules H_2O_2 are on order of 0.1-0.2 mM/L.

Taking into account only those components, we get δp_{osm}~0.7-1.4 kPa.

Most likely, as a result of exposure to plasma, the case $\sum n_{i,in} \ll \sum n_{i,out}$ is realized.

So, solution is *hypertonic*



When volume decreases, due to the outward flow of water

$\Sigma n_{i,in} \rightarrow \Sigma n_{i,out}$, and $\delta p_{osm} \rightarrow 0$

For real cells, due to the elasticity and stiffness

$\Sigma n_{i,in}(t) < \Sigma n_{i,iout}$, $\rightarrow \delta p_{osm}(t) < \delta p_{osm}(t=0)$

Even small changes in pressure can disrupt the mechanical properties of membranes, and, therefore, change the transport into or out of the cell.

The changes in the mechanical properties of membranes could be the reason for the selective effect of plasma on cells (for instance, the reason for selective apoptosis).

M.N. Shneider, M. Pekker, J.Appl.Phys. 123, 204701 (2018)

Cells charging

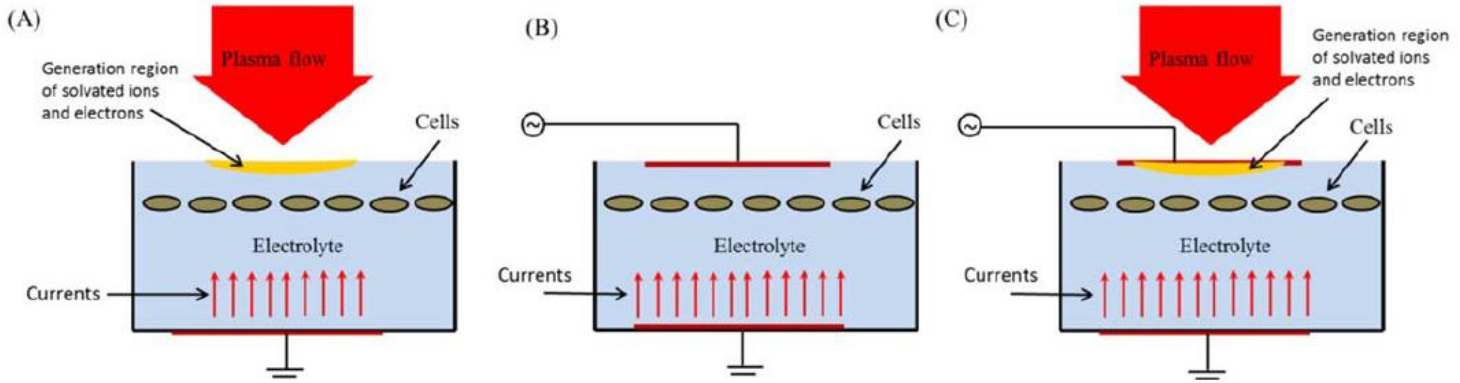
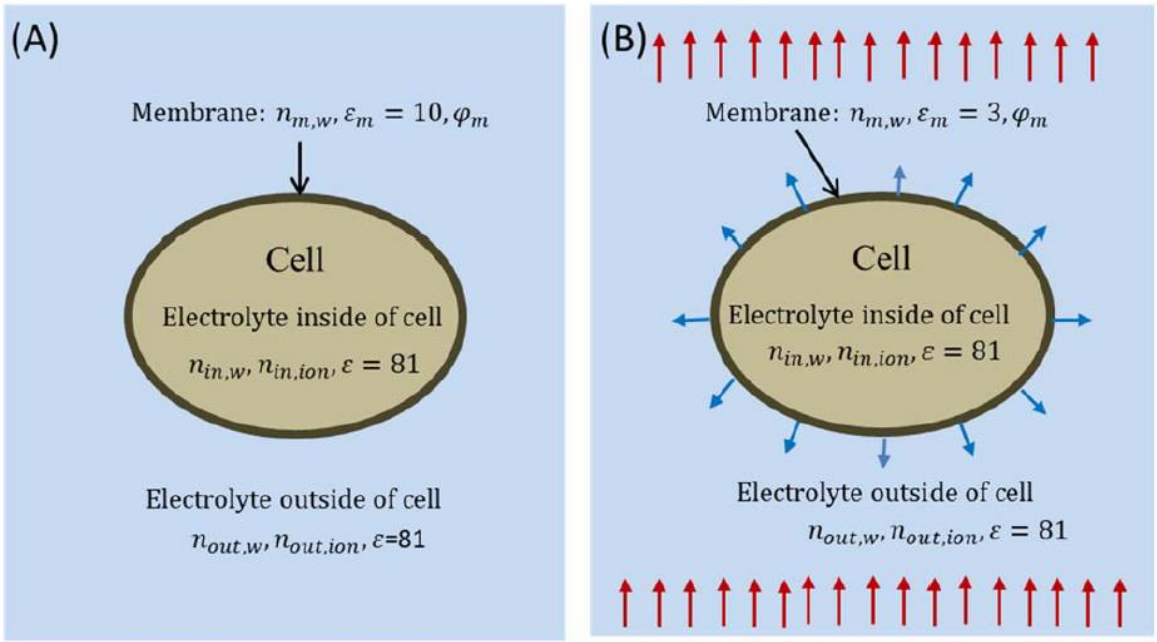
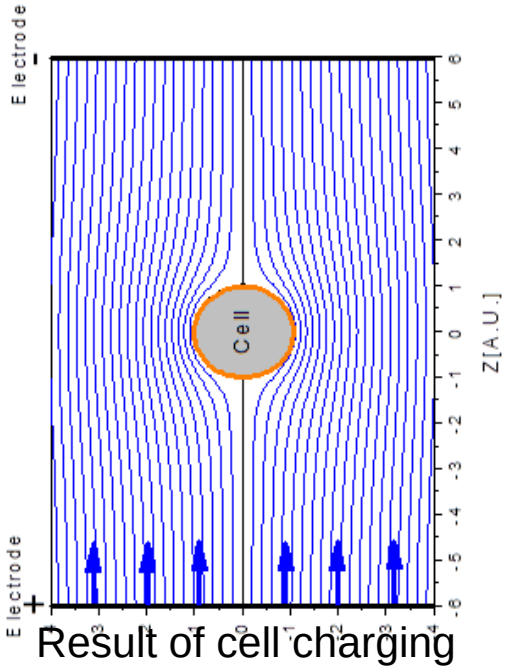


Figure 1. Schematics of the typical experimental setups with cell structures in saline in a Petri dish. (A) - plasma jet interacting with air-saline interface. (B)—currents induced by voltage applied to the electrodes. (C)—joint effect of applied voltage and plasma jet.





Result of cell charging

Figure 2. Cell in the physiological saline. (A)—no external source of weakly ionized plasma, (B)—an external source of nonequilibrium plasma interacts with saline. Red arrows indicate currents in physiological saline, and blue arrows indicate flow of water molecules through a membrane due to an increase in the density of hydrated ions near the cell.

M.N. Shneider, M. Pekker 2019 Plasma Res. Express 1 045001

Mathematical model

1. The cells are thin-walled spheres of radius *a* that are far enough from each other so that they can be considered independently of each other in the field of constant currents

2. The cell environment is an electrolyte with conductivity on the order of 1–5 Sm^{-1}.

3. The internal environment of the cell is also an electrolyte with conductivity close to the conductivity of the external medium.

4. The membrane is impermeable to ionic conduction currents, which charge the capacitance (bilayer lipid membrane), i.e., the current in the electrolyte is closed through the membrane capacitance by the displacement current, $c_m dU_m/dt$, where c_m is the membrane capacitance per unit area and U_m is the voltage on the membrane.

5.The cell membrane is charged with currents in the electrolyte until the additional surface charge accumulating on the membrane compensates for the radial field of the currents that charge the membrane.

M.N. Shneider, M. Pekker 2019 Plasma Res. Express 1 045001

The continuity equation for the currents

$$div\vec{j} = div(\sigma\vec{E}) = \sigma div(\vec{E}) = -\sigma\Delta\varphi = -\sigma\left(\frac{1}{r^2}\frac{\partial}{\partial r}r^2\frac{\partial\varphi}{\partial r} + \frac{1}{r^2\sin\theta}\frac{\partial}{\partial\theta}\sin\theta\frac{\partial\varphi}{\partial\theta}\right) = 0, \quad (1)$$

Since in our model the membrane is impermeable to ion currents, the membrane is charged until the stationary radial electric field is zero:

$$E_n = -\left.\frac{\partial\varphi}{\partial r}\right|_{r=a} = 0. \quad (2)$$

Far from the sphere, the current density is constant. Accordingly, the current, potential and the electric field are related by the relation:

$$\varphi_\infty = -E_0 r\cos(\theta) = -j_0 r\cos(\theta)/\sigma \quad (3)$$

The solution for (1) with boundary conditions (2), (3) is

$$\varphi_{r\geq a} = E_0 r\cos\theta\left(1 + \frac{a^3}{2r^3}\right)$$

and, at r = a: $\varphi_a = \frac{3}{2}E_0 a\cos\theta = \frac{3}{2\sigma}j_0 a\cos\theta$ $\qquad \varphi_a(0) = \frac{3}{2}E_0 a,$ at $\theta = 0$

For φ_a~100 mV, σ~1 S/m, a~10 μm: j_0~6 kA/m^2

In this case, the estimate of the transition time (characteristic charging time of the capacitor) is (at $c_m \approx 10^{-2}$ F/m^2)

$$\tau_{ch} \sim c_m\frac{|\varphi_a|}{j_n} = \frac{3}{2}\frac{c_m a}{\sigma} \sim 0.165\ \mu s$$

Additional voltage on membrane φ_a >~100 mV can lead to electroporation.

Where is selectivity?

M.N. Shneider, M. Pekker 2019 Plasma Res. Express 1 045001

Selective effect of plasma treatment on healthy and cancer cells

What is different in healthy and diseased cells?

Mechanical properties are different:

The measured module of the all-around compression and shear modulus for cancer cell are K_1=103.6 Nm^{-2} and K_2 =42.5 Nm^{-2} (**hepatocellular carcinoma cells (HCCs)**) and K_1=87.5 Nm^{-2} and K_2 =33.3 Nm^{-2}, respectively (**hepatocytes**)

Measured in: Zhang et al, World J. Gastroenterol. 8 243 (2002)

Two hypothesis: selective mechanical destruction and selective electroporation

I. Selective mechanical destruction initial ($\Delta p_{osm,c} = \Delta p_{osm,h}$)

All-around compression coefficient $K_{1,cancer} > K_{1,healthy}$, therefore **the cancer cell is more rigid** and **decreases in volume less than a healthy cell.**

So, less water flows from the cancer cell and therefore the pressure drop on the membrane of cancer cells becomes significantly greater than on the healthy cells, $\Delta p_{osm,c}(t) > \Delta p_{osm,h}(t)$, because of $(\Sigma n_{i,in})_c(t) > (\Sigma n_{i,in})_h(t)$

Therefore, for cancer cells, irreversible growth of pores and mechanical destruction of the membrane are more likely when the ionic composition of the physiological solution changes, caused by the action of the plasma source.

Plasma: $\Sigma n_{i,out}$. → initial ($\Delta p_{osm,c} = \Delta p_{osm,h}$) $K_{1,c} > K_{1,h}$, $\Delta V_c < \Delta V_h$, → $\Delta p_{osm,c}(t) > \Delta p_{osm,h}(t)$.

Selectivity, if $\Delta p_{m,c} > \Delta p_{crit}$ and $\Delta p_{m,h} < \Delta p_{crit}$, Δp_{crit} - critical destruction pressure difference

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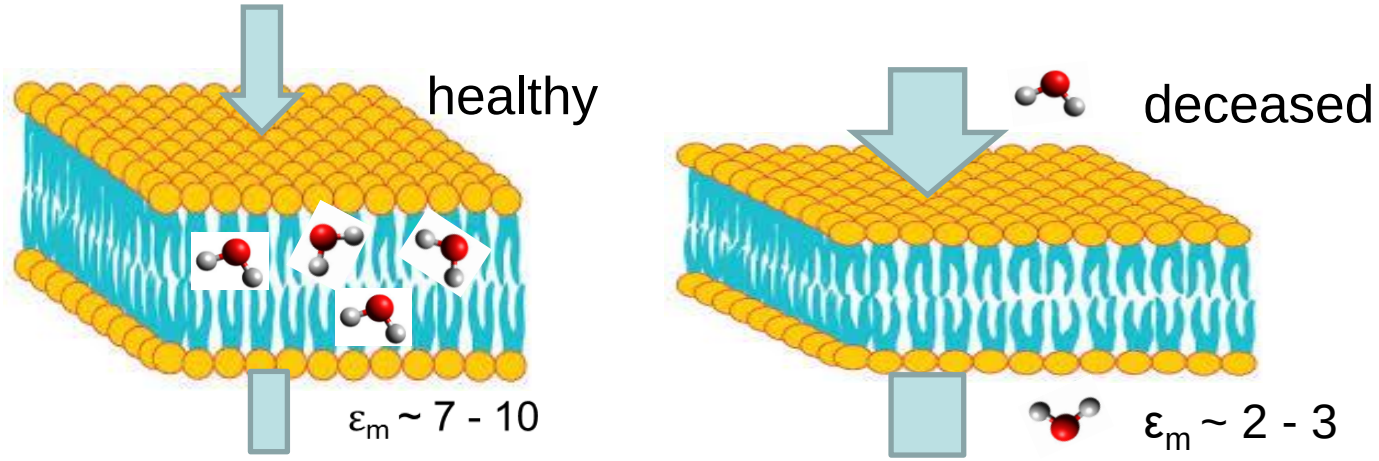
II. Selective charging.

The dielectric constant of the real phospholipid membrane in the cell, estimated by the experimental values of the capacitance of the membrane, is of the order of ε_m~7 – 10, while in the ideal phospholipid membrane, the relative dielectric permittivity is ε_m~2 – 3

Since the modulus of compression of membranes in diseased cells exceeds that of membranes in healthy cells, $K_{1,c} > K_{1,h}$ and, therefore, $\Delta p_{osm,c}(t) > \Delta p_{osm,h}(t)$ it should be expected that the decrease in the dielectric constant of membranes in diseased cells due to water displacement should be greater than the decrease in the dielectric constant in healthy cells.



If $c_{m,c} < c_{m,h}$, then $\varphi_{m,c} > \varphi_{m,h}$, $\varphi_m = Q_m / c_m$, $Q_m = S \int_0^{\tau_{ch}} j_n dt$

Therefore, with the same additional charge on the membrane, generated by currents induced in the intercellular solution, the voltage on the membrane and the probability of its electroporation are greater for cancer cells than for healthy ones.

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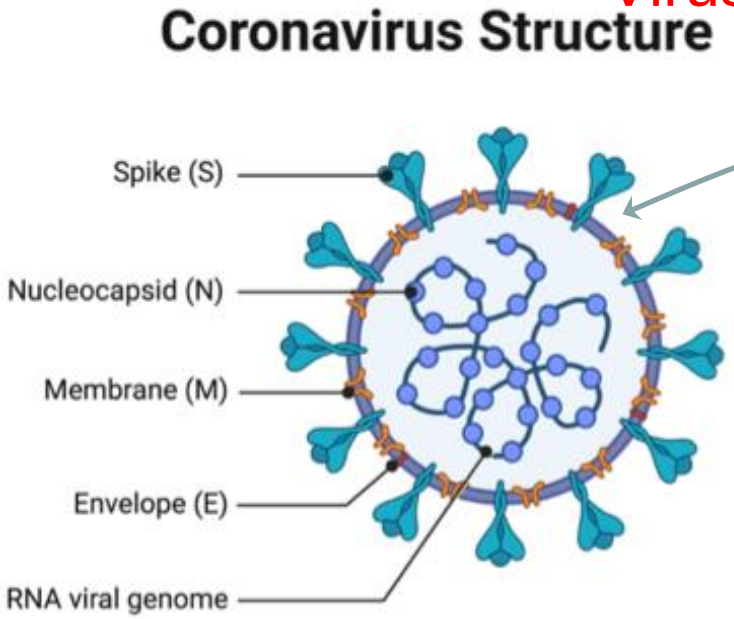
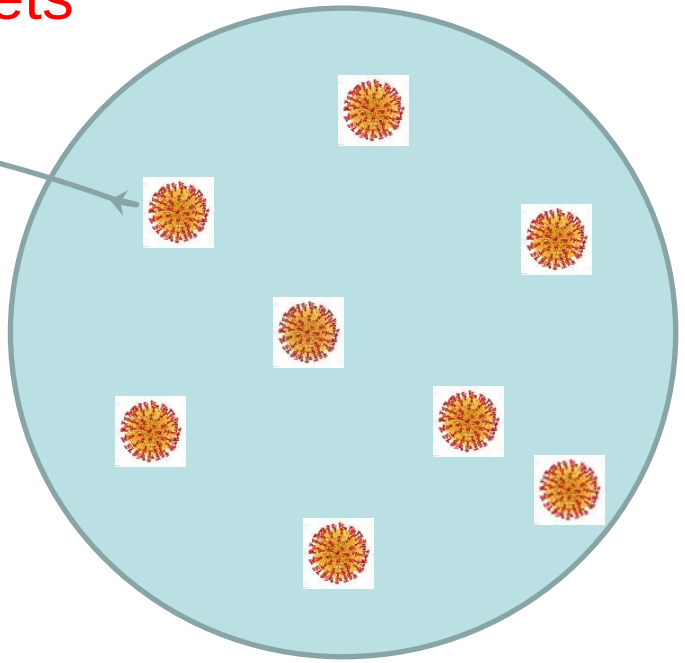
Viruses in droplets





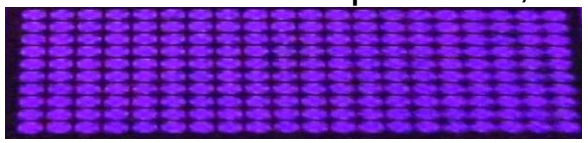
https://web.mit.edu/fnl/volume/324/king1_POP.html

Size: 50 -100 nm

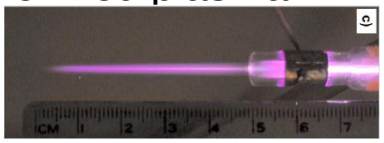
Liquid droplet (saliva) with viruses

R: 1-1000 µm

Treatment: non-equilibrium, weakly ionized plasma



https://pcrf.princeton.edu/



Shashurin, Keidar 2010

Ultraviolet

https://www.chinavasion.com/china/wholesale/personal-health-protection/disinfection-and-sterilization/household-uv-disinfection-stick-led-ste-pho-0mdarexj

The UV covers the wavelength range 100-400 nm and is divided into three bands:

UVA (315-400 nm) UVB (280-315 nm) UVC (100-280 nm).

UV: kill viruses (direct effect) + modify composition in droplets (formation H_2O_2 , etc)

Non-equilibrium plasma: change composition in droplet -> change osmotic pressure -> membrane destruction -> kill viruses **Exposure time:** $t \sim R^2/D$, D – diffusion coefficient for ions. At R~100 µm, t ~ 10 sec

Conclusions

•Nonequilibrium plasma generated by various sources in the air near the interface with physiological saline with cell or virus cultures changes the ionic and molecular composition of the solution. This leads to a change in the osmotic pressure drop on the membrane.

•Consideration of changes in osmotic pressure during interaction with plasma is necessary for quantitative and qualitative description, and optimization of various approaches to disinfection and sterilization.

•The osmotic pressure is not only an empirical qualitative parameter, but also a quantitative characteristic that can be used in choosing the optimal plasma source and in theoretical models and estimates.

•Taking into account the osmotic pressure makes it possible to predict the evolution of the cell shape and volume

•Our work **does not claim** that osmotic pressure changes constitute **the only mechanism** affecting cells in saline. Our work, rather, has identified a potential further physical mechanism that has relevance to plasma-induced effects on living cells.

•Intensive experimental and theoretical interdisciplinary research is needed

- measurements and modeling of ionic composition and its dynamics in a solution interacting with a plasma
- measurements osmotic pressure on cells
- visualization of modification of cell shape and size
- measurements of mechanical properties and dielectric constant of the membranes of healthy and diseased cells
- etc

Acknowledgements

This work was supported partially by the National Science Foundation (Grant No. 1465061)

Alexander Fridman	*Drexel University, Philadelphia, PA*
Igor Kaganovich	*Princeton Plasma Physics Laboratory, NJ*
Michael Keidar	*George Washington University, Washington, DC*
Mikhail Pekker	*Retired, Princeton, NJ*
Yevgeny Raitses	*Princeton Plasma Physics Laboratory, NJ*
Stephan Reuter	*Polytechnic University, Montreal, Canada*
Alexey Shashurin	*Purdue University, IN*
Svetlana Starikovskaya	*Ecole Polytechnique, Paris, France*
Andrey Starikovskiy	*Princeton University, NJ*
Shurik Yatom	*Princeton Plasma Physics Laboratory, NJ*

Thank You!!!

An example of hypotonic cell destruction

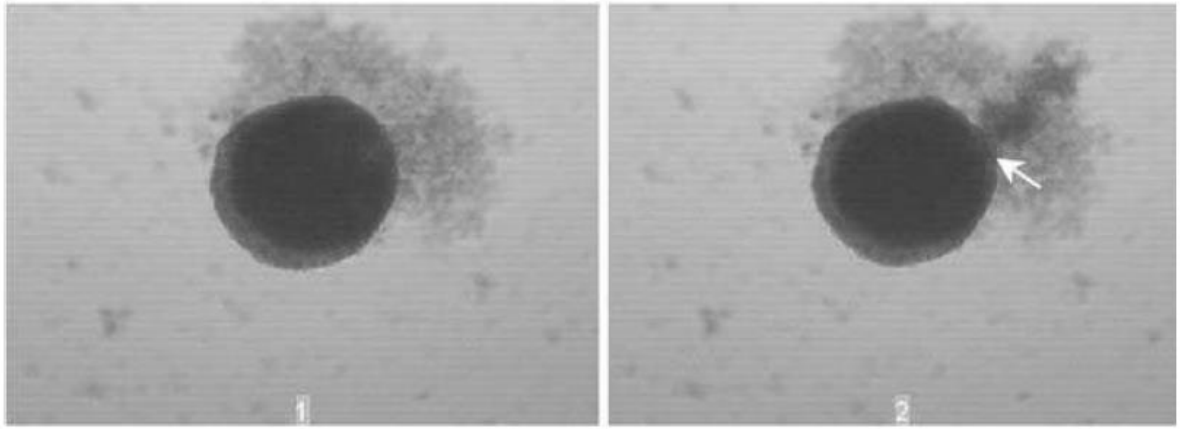


Fig. 4. A sequence of bursts in hydra aggregates. Picture one shows an aggregate with a diffuse cloud of expelled cells from the previous explosions, while picture two shows a cellular aggregate in the process of bursting. Arrow indicates the site of the burst.