Cold atmospheric plasmas as new anti-cancer therapy

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Cold atmospheric plasmas sources

- Dielectric barrier discharges
- Surface discharges
- Corona discharges
- DBD and RF plasma jets

Cold atmospheric plasmas sources

- No substantial heating of biological substrate
- Large surface or local treatment
- Scalable and tuneable (gap distance, gas flow, energy input, plasma chemistry)
- Portable and fulfil safety requirements
- Low operation cost
Mechanism of plasma action
Plasma interaction with liquid medium

\[
\begin{align*}
4 \text{ NO}^* + \text{ O}_2 + 2 \text{ H}_2\text{O} &\rightleftharpoons 4 \text{ H}^* + 4 \text{ NO}_2^- \\
4 \text{ NO}_2^- + \text{ O}_2 + 2 \text{ H}_2\text{O} &\rightleftharpoons 4 \text{ H}^* + 4 \text{ NO}_3^- \\
\text{NO}^* + \text{ NO}_2^- &\rightleftharpoons \text{ N}_2\text{O}_3 \\
\text{N}_2\text{O}_3 + \text{ H}_2\text{O} &\rightleftharpoons 2 \text{ NO}_2^- + 2 \text{ H}^* \\
\text{N}_2\text{O}_3 + \text{ O}_3 &\rightleftharpoons \text{ N}_2\text{O}_6 \rightleftharpoons 2 \text{ NO}_3^- \\
\text{NO}_3^- + \text{ NO}_2^- + \text{ H}_2\text{O} &\rightleftharpoons \text{ N}_2\text{O}_5 + \text{ H}_2\text{O} \\
\text{N}_2\text{O}_5 + \text{ H}_2\text{O} &\rightleftharpoons 2 \text{ H}^* + 2 \text{ NO}_3^- \rightleftharpoons 2 \text{ H}^* + 2 \text{ ONOO}^- \\
\text{N}_2\text{O} + \text{ O}^* &\rightleftharpoons \text{ CO}_2 \\
\text{O}^* + \text{ H}_2\text{O} &\rightleftharpoons 2 \text{ HO}^- \\
\text{HO}^- + \text{ O}_3 &\rightleftharpoons \text{ HOO}^- + \text{ O}_2 \\
2 \text{ HOO}^- &\rightleftharpoons \text{ H}_2\text{O}_2 + \text{ O}_2 \\
\text{HOO}^- &\rightleftharpoons \text{ H}^* + \text{ O}_2^- \\
\text{NO}^* + \text{ O}_2^- &\rightleftharpoons \text{ ONOO}^- \\
\text{NO}_2^- + \text{ HO}^- &\rightleftharpoons \text{ ONOOH} \\
\text{ONOOH} &\rightleftharpoons \text{ HNO}_3 \rightleftharpoons \text{ NO}_3^- + \text{ H}^* \\
\text{ONOO}^- + \text{ CO}_2 &\rightleftharpoons \text{ ONOOCCO}_2^- \\
\text{ONOOCCO}_2^- &\rightleftharpoons \text{ NO}_2^* + \text{ CO}_3^- \\
\end{align*}
\]
Plasma produces long living ROS in tissues

Dobrynin et al. 2011

- Role of electric field in diffusion of ROS

Szili et al. 2014

Dobrynin et al. 2011
Cancer treatment therapies

- Surgical excision
- Radiotherapy
- Chemotherapy
- Immunotherapy
- Photodynamic therapy
- Electro-chemotherapy
Nanosecond DBD

Pulser → Signal Generator
Monochromator → iCCD

Bottom view
Side view

30 ns
3-10 kV
1 Hz - 1 kHz
Nanosecond DBD

![Diagram of Nanosecond DBD system]

- BCS
- Pulser
- Signal Generator
- Monochromator
- ICCD
- Objective
- OF

![Graph showing pulse energy and intensity]

- Pulse energy, mJ/ns
- Incident pulse
- Reflected pulse
- 20 mJ/pulse

![Graph showing voltage and time]

- Voltage, kV
- Time, μs
- BCS signal
- 250 ns
- 800 ns

![Graph showing intensity vs. wavelength]

- Intensity, a.u.
- Wavelength, nm
- Experiment
- 380 K
Nonuniform discharge => nonuniform treatment?

Deposited charges => uniform streamers repartition

[U Kogelschatz, 2002]
Nude mice skin treatment *in vivo*
Plasma treatment of living cells *in vitro*

 HV cell detachment => death (anoikis?)

Trypan Blue assay

Control

10^4 pulses
Plasma treatment of living cells *in vitro*

Electrostatic repulsion due to the deposited charges overcomes surface tension and gravity.

$10^4$ pulses

Control
Plasma treatment of living cells *in vitro*

**10^4** pulses

Uniform treatment !!
Protocole

- Normal thyrocyte HTori-3 or Melanoma A375 $1.5 \times 10^5$ cells seeded in Ø35mm Petri dish 2 days prior to treatment (J-2)
- Cultured in 2 ml of DMEM +10% SFV
- Washed with PBS Mg$^{2+}$Ca$^{2+}$ at 37C
- Supplied with 1.5 ml of PBS Mg$^{2+}$Ca$^{2+}$
- Treated at 300 Hz for 1500, 3000, 5000, 10000 pulses
- Incubated for 60 min in treated PBS
- Analysed at H1, H24, H72
### Cell adhesion assay

<table>
<thead>
<tr>
<th></th>
<th>CTRL</th>
<th>1500</th>
<th>3000</th>
<th>5000</th>
<th>10000</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>H1</strong></td>
<td><img src="image1.png" alt="Images" /></td>
<td><img src="image2.png" alt="Images" /></td>
<td><img src="image3.png" alt="Images" /></td>
<td><img src="image4.png" alt="Images" /></td>
<td><img src="image5.png" alt="Images" /></td>
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<tr>
<td><strong>H24</strong></td>
<td><img src="image6.png" alt="Images" /></td>
<td><img src="image7.png" alt="Images" /></td>
<td><img src="image8.png" alt="Images" /></td>
<td><img src="image9.png" alt="Images" /></td>
<td><img src="image10.png" alt="Images" /></td>
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<tr>
<td><strong>H72</strong></td>
<td><img src="image11.png" alt="Images" /></td>
<td><img src="image12.png" alt="Images" /></td>
<td><img src="image13.png" alt="Images" /></td>
<td><img src="image14.png" alt="Images" /></td>
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- No visible effect at early times (H1)
- Lose of adhesion (death) in dose dependent manner (H24)
- Proliferation of survived cells (H72)
Air plasma in contact with water and tissues

\[ \text{H}_2\text{O}_2 \text{ production/diffusion in the porcine skin} \]

[Bruggeman 2009]

[Dobrynin 2011]
Dosage of plasma produced H$_2$O$_2$

Ampex® Red (10-acetyl-3,7-dihydroxyphenoxazine)

Horseradish peroxidase (HRP)

- Selective and sensitive (nmol/L)
- Fluorescent signal intensity at 595 nm
H$_2$O$_2$ mediates cell killing

The H$_2$O$_2$ is the main effector of plasma action, however alone does not seem to explain the plasma toxicity => electric field, pH, UV other ROS?
Morphological aspect

H2

CTRL

100 µM

10^4p

10^4p + CAT
Extracellularly produced H$_2$O$_2$ is ‘consumed’ by the cells
DNA DSB

\[ \text{H}_2\text{O}_2 \xrightarrow{\text{uv}} 2\text{OH}^\bullet \]

\[ \text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^\bullet + \text{OH}^- \quad \text{(Fenton)} \]

\[ \text{O}_2^- + \text{H}_2\text{O}_2 \rightarrow \text{^1O}_2 \left( ^1\Delta_g \right) + \text{OH} + \text{OH}^- \quad \text{(Haber-Weiss)} \]

- \( \text{H}_2\text{O}_2 \) cannot directly produce DNA DSB
Conclulsion

• Liquid medium allows ‘uniformisation’ of plasma treatment
• Threshold in cell adhesion (viability) depending on number of successive treatments and applied HV pulses
• H$_2$O$_2$ is the major effector (but not the only!) of plasma action
• DNA DSB is correlated with extracellular concentration of H$_2$O$_2$
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