Mechanistic Insights to Cold Plasma Functionalised Liquids: Antimicrobial efficacy and Interactions with Processing and Storage Conditions

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Plasma
Plasma – the 4th state of matter
Plasma treatment of liquids

Plasma activated liquids (PAL)

Plasma functionalized liquids (PFL)

Plasma treated liquids (PTL)
Plasma functionalized liquids (PFL)

Chemical changes:
- ONOO⁻
- HONO
- NO₃⁻
- OONOO⁻
- H₂O₂
- NO₂⁻

Biological activity:

Applications:
- Wounds
- Cancer treatment
- Surface decontamination
- Food
- Sanitizing agent
- Agriculture
- Waste/wastewater

Biological activity:

ONOO- → HONO → pH ↓ → NO₃⁻ → OONOO⁻ → H₂O₂ → NO₂⁻
What happens during plasma treatment of liquids?

Schematic diagram of formation of reactive species in liquid
Different plasma functionalized liquids

Plasma device + treatment parameters

Liquid composition

![Images of plasma devices and liquid bottles]

- Reactive species
- Unknown
Plasma functionalized liquids based on discharge in air

DBD-120 system

RSS system
Reactive chemical species in plasma functionalized liquids

- Chemistry:
  - pH, ORP, conductivity
  - Detection of long-lived ROS/RNS
    - $\text{H}_2\text{O}_2$: TiOSO$_4$
    - Oxidative species (peroxides, HNO$_2$): KI (buffered/non-buffered)
    - NO$_2^-$: Griess
    - NO$_3^-$: Dimethylphenol

System parameters
- Plasma devices
- Treatment

Process parameters
- Storage
- Temperature

Liquid parameters
- Composition
- Buffering

Tsoukou et al. (2018), *Plasma medicine*
Niquet, Boehm et al. (2017), *Plasma Processes and Polymers*
The DBD120 system

Voltage: 0-120kV
Frequency: 50 Hz
Gap: 22mm

Liquid composition

<table>
<thead>
<tr>
<th></th>
<th>Buffered (KH$_2$PO$_4$/K$_2$HPO$_4$)</th>
</tr>
</thead>
</table>
|                | -                                    | +
| Saline (NaCl)  | -/-                                  | -/+    |
|                | +/- H$_2$O                            | +/+ PB |
|                | +/- S                                | +/+ PBS|

Tsoukou et al. (2018), Plasma medicine
Chemical characterization

- **pH**
  - PAW
  - PAPBS
  - PAS
  - PAPB

- **Hydrogen Peroxide**
  - PAW
  - PAPBS
  - PAS
  - PAPB

- **Nitrites**
  - PAW
  - PAPBS
  - PAS
  - PAPB

- **Nitrates**
  - PAW
  - PAPBS
  - PAS
  - PAPB

PAW: Plasma activated Water
PAPBS: Plasma activated PBS
PAS: Plasma activated Saline
PAPB: Plasma activated PB

Evanthia Tsoukou

PAW: Plasma activated Water
PAPBS: Plasma activated PBS
PAS: Plasma activated Saline
PAPB: Plasma activated PB
The RSS plasma system

Dr. Peng Lu

Spark (SD)  
Glow (GD)

ROS-rich  
$\text{H}_2\text{O}_2$, $\text{NO}_3^-$

RNS-rich  
$\text{NO}_3^-$, $\text{NO}_2^-$

Lu et al. (2017) Plasma Processes and Polymers
Different plasma systems – different chemistry

<table>
<thead>
<tr>
<th></th>
<th>PTW-MW</th>
<th>PTW-DBD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Input energy</td>
<td>90-920 W min</td>
<td>300-3500 W min</td>
</tr>
<tr>
<td>Nitrous acid</td>
<td>2-12 mM</td>
<td>Not detected</td>
</tr>
<tr>
<td>Nitrite</td>
<td>2-20 mM</td>
<td>Not detected</td>
</tr>
<tr>
<td>Nitrate</td>
<td>1-25 mM</td>
<td>0.1-0.8 mM</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>Not detected</td>
<td>0.02-0.4 mM</td>
</tr>
<tr>
<td>Contact time for microbial inactivation</td>
<td>~1 min</td>
<td>~60 min</td>
</tr>
</tbody>
</table>

Collaboration between TU Dublin and the INP Greifswald:

Comparison of chemical composition and antimicrobial efficacy of plasma activated water

Effects of PFLs on prokaryotic cells

- **Antimicrobial activity**
  - **Microbial target**
  - **Temperature stability**
  - **Liquid composition**
  - **Storage stability**
  - **pH dependency**

<table>
<thead>
<tr>
<th>PAL</th>
<th>Antimicrobial Efficacy (E.coli/S.aureus)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAW</td>
<td>Strong/Strong</td>
</tr>
<tr>
<td>PAPBS</td>
<td>Weak/Strong</td>
</tr>
<tr>
<td>PAS</td>
<td>Strong/Strong</td>
</tr>
<tr>
<td>PAPB</td>
<td>Median/Median</td>
</tr>
</tbody>
</table>

- **E.coli S15**
- **S. aureus S15**

Antimicrobial activity and stability of PFLs

![Graphs showing antimicrobial activity and stability of PFLs on day 1 and day 2.](image)

**Day 1**
- **PAW-Day 1**
- **PAS-Day 1**
- **PAPBS-Day 1**
- **PAPB-Day 1**

**Day 2**
- **PAPBS-Day 2**
- **PAPB-Day 2**

* log cfu/ml vs. Plasma Treatment Time (min) for E.coli and S.aureus 60min.
The role of pH in PFL antimicrobial activity

Neutralization of pH

- H2O
- H2O + 4.5xPBS
- PAW 5min
- PAW 5min + 4.5xPBS

Reduction of pH

- PBS
- PB + HCl
- PAPB 5min
- PAPB 5min + HCl

- NaCl
- NaCl + 4.5xPBS
- PAS 5min
- PAS 5min + 4.5xPBS

- PBS
- PBS + HCl
- PAPBS 5min
- PAPBS 5min + HCl
Antimicrobial activity and stability (RSS system)

PFW:
Spark (S) 5, 10, 15min
Glow (G) 5, 10, 15min

**Day 1**

- **E. coli 60 min**
  - CTL
  - S5
  - S10
  - S15
  - G5
  - G10
  - G15

- **S. aureus 60 min**
  - CTL
  - S5
  - S10
  - S15
  - G5
  - G10
  - G15

**1 Week**

- **E. coli 60min**
  - CTL
  - S5
  - S10
  - S15
  - G5
  - G10
  - G15

- **S. aureus 60min**
  - CTL
  - S5
  - S10
  - S15
  - G5
  - G10
  - G15
Temperature stability

Bactericidal effects retained after prolonged storage at -80, -150°C
Retention of antimicrobial efficacy at high temperature

Enhanced antimicrobial efficacy at high temperature and pressure?
Stability of PFL

Why does it matter?

- Off-site production
- Storability
- Applications in fumigation/vapourization
- Understanding chemistry and secondary reactions
Safety of plasma activated liquids

**Short and long-term safety**

**Cytotoxicity testing**
- Mammalian cell models
- *Galleria mellonella*

**Genotoxicity testing**
- Mammalian cell model (HPRT assay)
- Bacterial cell model (AMES test)

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**Long-term exposure – mutagenic effects**

<table>
<thead>
<tr>
<th>PBS</th>
<th>Day in culture</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>------</td>
<td>----</td>
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<tr>
<td><strong>Control</strong></td>
<td></td>
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<tr>
<td>A</td>
<td>-</td>
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<tr>
<td>B</td>
<td>-</td>
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<tr>
<td>C</td>
<td>nd</td>
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<tr>
<td>1 min</td>
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<tr>
<td>A</td>
<td>nd</td>
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<td>B</td>
<td>nd</td>
</tr>
<tr>
<td>C</td>
<td>nd</td>
</tr>
<tr>
<td>5 min</td>
<td></td>
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<tr>
<td>A</td>
<td>nd</td>
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<tr>
<td>B</td>
<td>nd</td>
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<tr>
<td>C</td>
<td>nd</td>
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<tr>
<td>10 min</td>
<td></td>
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<tr>
<td>A</td>
<td>nd</td>
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<tr>
<td>B</td>
<td>nd</td>
</tr>
<tr>
<td>C</td>
<td>nd</td>
</tr>
</tbody>
</table>

**Increasing rate of mutations over time**

**Highest occurrence of mutations**
H$_2$O$_2$ in PFL contributes to cytotoxic effects
other reactive species are involved

Differences in cytotoxic effects of biomolecule solutions
not a result of different H$_2$O$_2$ concentrations
Plasma-treated biomolecule solutions – mutagenic potential

Cell culture medium supplemented with 10% (v/v) biomolecule solution (in DMEM-F12) at each sub-culturing over 34 days
In vivo toxicity testing

_Galleria melonella_, injection model

Dead larvae

![Image of dead larvae]

![Graphs showing % survival for BSA, Arachidonic Acid, Glucose, and Cholesterol]
Toxicity testing of a plasma treated food model

- lettuce broth
  Plasma treatment:
  0, 1, 5, 10 min

Short-term in vitro toxicity

Long-term in vitro mutagenicity

- PFL can be
  - Storable (limited shelf-life at RT, extended shelf-life in frozen state)
  - Controllable (chemistry - device, discharge, liquid parameters)
  - Stable (temperature)
  - Modifiable? (influencing secondary reactions)

- Antimicrobial efficacy depends on
  - Concentration and type of ROS/RNS
  - Low pH
  - Contact time
  - Microbial species
Outlook - Challenges and opportunities

- **Engineering**
  - Selectivity
  - Scalability (Process assurance, reproducibility)
  - Storability

- **Application**
  - Versatility
  - Mode of application
    - Washing
    - Vapourization/fumigation
    - Freezing

- **(Bio)Chemistry**
  - Reactive species
  - Molecular modifications
  - Biochemical/cellular mechanisms
Tailoring plasma functionalized liquids for specific applications?

<table>
<thead>
<tr>
<th>Plasma device + treatment parameters</th>
<th>liquid composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>A B G</td>
<td>A B C G H I</td>
</tr>
</tbody>
</table>

Defined effect
INP Greifswald
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Dr Uta Schnabel
Rijana Niquet
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PrinciPAL - “Harnessing plasma-activated liquids (PAL) for biomedical applications”

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Thank you!

Questions?

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