Ambient Gas Plasma: From Infection Control to Medical Therapy

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Natural *Ionized Gas* Plasmas

- **Lightning**
- **Aurora**
- **Solar Corona**
- ‘Whirlpool galaxy’ M51; plasma emission and magnetic field lines
ITER: International Thermonuclear Experimental Reactor: plasma fusion for electric power generation
‘Low Temperature’ Plasmas “In the Kitchen”

* Plasma-processed microelectronics

Plasma TV

Plasma ion-implanted artificial hip


- Plasma TV
- Plasma-coated jet turbine blades
- Plasma-manufactured LEDs in panel
- Diamondlike plasma CVD eyeglass coating
- Plasma ion-implanted artificial hip
- Plasma laser-cut cloth
- Plasma HID headlamps
- Plasma-produced H₂ in fuel cell
- Plasma-aided combustion
- Plasma muffler
- Plasma ozone water purification
- Plasma-deposited LCD screen
- Plasma-deposited silicon for solar cells
- Plasma-deposited microelectronics
- Plasma-processed silicon for pharmaceutical production
- Plasma-treated polymers
- Plasma-treated textiles
- Plasma-treated heart stent
- Plasma-deposited diffusion barriers for containers
- Plasma-sputtered window glazing
- Compact fluorescent plasma lamp
Plasma Light Sources

Gas discharge lamps

Plasma displays: one pixel
Hall Thruster: Plasma Propulsion

Spacecraft and satellite propulsion
Plasma Airflow Actuators


V \sim 0.4 \text{ m/s}

V \sim 3 \text{ m/s}
Plasmas used to process semiconductors to make integrated circuits (ICs) – Nanoelectronics has replaced microelectronics: sub-10 nm critical dimensions
**Ambient gas** plasma sources for healthcare applications: direct and indirect

- **Direct exposure**
  - charged particles
  - electric field/charge accumulation
  - reactive neutrals
  - UV photons

- **Indirect exposure**
  - reactive neutrals (long-lived)
  - UV photons

Plasma Needle


- Frequency: RF (13.56MHz)
- Voltage: 200-400 V\(_{pkpk}\)
- He flow rate: \(~1\ \text{slpm (}\ Re_d < 100\)\)
- Power consumption: \(~1\ \text{W}\)
- Distance to sample: 1-5 mm
Plasma needle: Bactericidal effects


S. mutans
• bacteria
• anaerobic
• oral cavity

0.3 m/s light intensity 1.0 m/s

alive inactivated

5 mm
Strategy: hybrid approach

Visible image

Plasma-flow (2D model)

Plasma chemistry (1D model)

Classical molecular dynamics simulation
Fluid model: governing equations

Neutral Gas flow (He, N₂)

\[ \nabla \cdot (\rho \mathbf{u}) = 0, \quad \nabla \cdot (\rho \omega_{\text{air}} \mathbf{u} - \rho D \nabla \omega_{\text{air}}) = 0 \quad \text{(mass conservation)} \]

\[ \nabla \cdot (\rho \mathbf{u} u_i) = -\nabla p - \nabla \cdot \mathbf{\tau} + \sum q_i n_i \mathbf{E} \quad \text{(momentum conservation)} \]

\[ \nabla \cdot (-\lambda \nabla T + \mathbf{u}_c T) = \Phi + \sum q_i \Gamma_i \mathbf{E} + Q_{el} \quad \text{(energy conservation)} \]

Plasma dynamics

\[ \frac{\partial n_i}{\partial t} + \nabla \cdot \Gamma_i = S_i \]

\[ \Gamma_i = \text{sgn}(q_i) n_i \mu_i \mathbf{E} - D_i \nabla n_i + n_i \mathbf{u} \quad \text{(chg’d particle momentum)} \]

\[ \frac{\partial (n_e \mathbf{\varepsilon})}{\partial t} + \nabla \left( \frac{5}{3} \mathbf{\varepsilon} \mathbf{\Gamma}_e - \frac{5}{3} n_e D_e \nabla \mathbf{\varepsilon} \right) = -\mathbf{\Gamma}_e \cdot \mathbf{E} - Q \quad \text{(electron energy)} \]

\[ \varepsilon_0 \nabla \cdot \mathbf{E} = \sum q_i n_i \]

Chemistry model: from 2D to 1D

Mole fraction of air (log scale)

Killing pattern

on-axis

off-axis

1D plasma model
Chemistry model: chemical reactions

46 species

**negative particles:** e, O\(^{-}\), O\(_2\)\(^{-}\), O\(_3\)\(^{-}\), O\(_4\)\(^{-}\), H\(^{-}\), OH\(^{-}\)

**positive particles:** He\(^{+}\), He\(_2\)\(^{+}\), N\(^{+}\), N\(_2\)\(^{+}\), N\(_3\)\(^{+}\), N\(_4\)\(^{+}\), O\(^{+}\), O\(_2\)\(^{+}\), O\(_4\)\(^{+}\), NO\(^{+}\), N\(_2\)O\(^{+}\), NO\(_2\)\(^{+}\), H\(^{+}\), OH\(^{+}\), H\(_2\)O\(^{+}\), H\(_3\)O\(^{+}\)

**neutrals:** He, He\(^{*}\), He\(_2\)\(^{*}\), N, N\(^{*}\), N\(_2\), N\(_2\)\(^{*}\), N\(_2\)\(^{*}\), O, O\(^{*}\), O\(_2\), O\(_2\)\(^{*}\), O\(_3\), NO, N\(_2\)O, NO\(_2\), NO\(_3\), H, H\(_2\), OH, H\(_2\)O, HO\(_2\), H\(_2\)O\(_2\)

214 elementary reactions

- 21 electron impact excitation/ionization/dissociation reactions
- 20 Penning and associative ionization reactions
- 26 electron recombination/attachment reactions
- 65 charge transfer reactions
- 51 ion recombination reactions
- 31 neutral-neutral reactions
Chemistry model: phase-averaged flux on surface

On-axis:

Flux \(10^{19} \text{ m}^{-2} \text{s}^{-1}\)

Off-axis:

Reactive neutrals

\(e, \text{He}_2^+, \text{N}_2^+, \text{O}_2^+, \text{H}_2\text{O}^+, \text{N}_2^+, \text{O}_2^+, \text{H}, \text{N}, \text{OH}, \text{NO}, \text{O}_2^*\)
Model validation: O atom measurement

- **TALIF**: two photon absorbed laser induced fluorescence
- collaboration with Ruhr-Universität Bochum (Germany)

Model validation: measured O atom density

Typical chemistries produced by air plasmas (T \sim 300\text{K})
Immune System: Innate and Adaptive

**Innate immunity**
- Epithelial barriers
- Phagocytes
- Complement
- NK cells

**Adaptive immunity**
- B lymphocytes → Antibodies
- T lymphocytes → Effector T cells

<table>
<thead>
<tr>
<th>Hours</th>
<th>Days</th>
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<tr>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>12</td>
<td>5</td>
</tr>
</tbody>
</table>

Time after infection
Innate Immune System: Inflammation

Innate, inflammation-based immunity is first line of defence against invading pathogens; adaptive immune system follows.

Phagocytic cells, e.g. macrophages and neutrophils, are the main innate inflammatory response agents.

- **ROS and RNS** are the most important micromolecules in innate immune system
- cannot discriminate between host and invader
- acquired immune system: *exquisitely selective*
Role of ROS/RNS in Immune System Response to Infection

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Neutrophils</th>
<th>Macrophages</th>
<th>Dendritic cells</th>
<th>Natural killer cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Function</td>
<td>Phagocytosis</td>
<td>Phagocytosis</td>
<td>Antigen presentation</td>
<td>Lysis of viral-infected cells</td>
</tr>
<tr>
<td></td>
<td>Reactive oxygen and nitrogen species</td>
<td>Inflammatory mediators</td>
<td>Costimulatory signals</td>
<td>Interferon</td>
</tr>
<tr>
<td></td>
<td>Antimicrobial peptides</td>
<td>Antigen presentation</td>
<td>Reactive oxygen species</td>
<td>Macrophage activation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reactive oxygen and nitrogen species</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cytokines</td>
<td>Interferon</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Complement proteins</td>
<td>Cytokines</td>
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</tr>
</tbody>
</table>

Figure 3-12
Kuby IMMUNOLOGY, Sixth Edition
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ROS/RNS utilized in innate immune system response
Antimicrobial species generated from oxygen and nitrogen

**Reactive oxygen species (ROS)**
- O$_2^-$ (superoxide anion)
- OH$^-$ (hydroxyl radical)
- H$_2$O$_2$ (hydrogen peroxide)
- ClO$^-$ (hypochlorite anion)

O$_2$ (Oxygen) $\rightarrow$ O$_2^-$ (Superoxide anion) $\rightarrow$ H$_2$O$_2$ (Hydrogen peroxide) $\rightarrow$ HClO$^-$ (Hypochlorite)

NADPH phagosome oxidase $\rightarrow$ Superoxide dismutase

**Reactive nitrogen species (RNS)**
- NO (nitric oxide)
- NO$_2$ (nitrogen dioxide)
- ONOO$^-$ (peroxynitrite)

NO (Nitric oxide) $\rightarrow$ ONOO$^-$ (Peroxynitrite) $\rightarrow$ NO$_2$ (Nitrogen dioxide)

Figure 3-13
Kuby IMMUNOLOGY, Sixth Edition
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Further Roles of ROS and RNS

1. Signaling: neurotransmission, phagocyte activation, iron metabolism, cell proliferation, and apoptosis

2. Regulation of vascular tone: NO in cardiovascular system and vasodilation

3. Host-tissue injury: certain kinds of pneumonia, encephalitis, etc.

4. Control of inflammation: ameliorates tissue damage.

Fang, Nature Reviews Microbiology, 2004
One Major Thrust for Plasma Healthcare: Infection Control

• Global factors promoting spread of infectious disease
• Hospital-acquired infections
• Growing challenge of antimicrobial resistance

Special challenges of infection control in the developing world.

Opportunity for plasma technology to have significant impact:

CHEAP, SIMPLE, EFFECTIVE
Global Factors Promoting Spread of Infectious Disease

• globalization: rapid movement of people, food, microbes

• explosive population growth, rise of large cities, coupled with poverty, urban migration and limited public health facilities

• intensive/concentrated livestock industry

• global climate change disrupting ecosystems

• antimicrobial resistance: inexorable rise in number of resistant microbes limits use of traditional infection control (e.g. antibiotics)

• emerging threat of bioterrorism
Major Problem: Hospital-Acquired Infections: HAI, or ‘Nosocomial’ Infections

2002 in US: 1.7 million HAIs & 99,000 deaths

Catheter-associated urinary tract infections. CAUTI is a common nosocomial infection, with an estimated 1 million cases in the US each year.

Routes of entry of pathogens to catheterized urinary tract

Major Problem: Hospital-Acquired Infections: HAI, or ‘Nosocomial’ Infections

2002 in US: 1.7 million HAIs & 99,000 deaths

Catheter-related bloodstream infections. CRBSI occurs about 250,000 times per year in US hospitals.

Surgical site infections. This accounts for a significant number of HAIs.

Ventilator-associated pneumonia (VAP). VAP is thought to occur on up to 25% of all people who are on mechanical ventilation for at least 48 hours. Of these, morbidity rates are among the highest of all forms of HAI.
In some diseases, resistance is developing for virtually all currently available drugs: an post-antibiotic era may be coming!

But modern medicine relies on invasive procedures, and antimicrobials are essential in current practice to avoid subsequent, often inevitable infections.
Pharmaceutical companies not developing new drugs!

**Figure 2:** The number of new systemic antibiotic agents has declined since 1980, and most (75%) of these drugs are in two classes, beta-lactams and quinolones.
Elements of *Plasma-Assisted Infection Control*

1. Plasma used to sterilize/disinfect environment
   - air and/or aerosol-based microbes
   - water-based microbes
   - microbes on objects/surfaces (‘fomites’)

2. Plasma used to sterilize/disinfect in/on devices
   - medical device surface (e.g. surgical)
   - catheter extra-luminal or intra-luminal (e.g. vs. biofilm)
   - implant device sterilization

3. Plasma on skin/wounds/burns or during invasive procedure
   - hand hygiene; pre-/post-invasive procedure
   - coupled with immune system/wound repair
   - *key role of reactive oxygen/nitrogen species ROS/RNS*
Special Challenges of HAIs in LRCs

L. Raca, J. Hospital Infec, 2009; V. Rosenthal et al., AJIC, 2008

- 12 million ID deaths occur globally: 95% in LRCs

- Infection rates in LRC hospitals ~ 3-5 times higher than in industrialized countries

- HAIs in 5-10% of admissions to acute care hospitals in industrialized countries, but in >40% of hospitalizations in developing countries in Asia, Latin America and Africa.

- In ICUs of LRCs: 66% of admitted patients develop a HAI; ~25% of admitted patients in ICUs in industrialized countries develop HAI (w/25% mortality)

- VAP mortality rates ~ 16% to 94% in LRCs
### Special Challenges of HAIs in LRCs

**L. Raca, J. Hospital Infec, 2009**

<table>
<thead>
<tr>
<th>Country</th>
<th>Type of study/unit</th>
<th>HCAI rate (%)</th>
<th>Year</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentina</td>
<td>Multicentre adult ICU</td>
<td>27.0</td>
<td>2003</td>
<td>Rosenthal</td>
</tr>
<tr>
<td>Brazil</td>
<td>Multicentre adult ICU</td>
<td>29.6</td>
<td>2006</td>
<td>Salomao</td>
</tr>
<tr>
<td>India</td>
<td>Multicentre adult ICU</td>
<td>12.3</td>
<td>2005</td>
<td>Mehta</td>
</tr>
<tr>
<td>Mexico</td>
<td>Multicentre adult ICU</td>
<td>24.4</td>
<td>2006</td>
<td>Ramirez</td>
</tr>
<tr>
<td>Saudi Arabia</td>
<td>Newborn ICU</td>
<td>35.8</td>
<td>2002</td>
<td>Al-Ghamdi</td>
</tr>
<tr>
<td>Tanzania</td>
<td>Adult medical ICU</td>
<td>40.0</td>
<td>2003</td>
<td>Gosling</td>
</tr>
<tr>
<td>Kosova</td>
<td>Adult ICU</td>
<td>68.7</td>
<td>2006</td>
<td>Spahija</td>
</tr>
<tr>
<td>Turkey</td>
<td>Neurology ICU</td>
<td>88.9</td>
<td>2005</td>
<td>Cevik</td>
</tr>
</tbody>
</table>

ICU, intensive care unit.
Plasma Device Proposed: 1. Handheld Air DBD

Blum/SPSP 2010: Ambient Gas Plasma: A sustainable and viable tool for infection control in the developing world

- Air plasma creates acidic (pH ~ 2.5), highly oxidizing aqueous solution
Air Plasma-Water Treatment: Preliminary Results

Tesla coil, 1 mm gap from liquid surface; 1 mL of liquid in a glass vial (about 8 mm liquid height) containing e coli
Advantages of Plasma-Based Devices for LRCs?

Devices are inexpensive, simple to employ, and use little electricity.

Obvious applications include instrument sterilization and:

- catheter/surgical site incision wound disinfection/sterilization/antisepsis, including dental care;
- wound healing and other uses a bonus

Device manufacture/repair in LRCs appear feasible.

Also, advantages in emergencies or natural disasters where secondary infection control is difficult due to lack of supplies
Ambient Gas Plasma Therapeutics

- Surgery; cell/tissue removal, coagulation
- Wound healing, blood clotting, \textit{in-vivo} anti-microbial action
- Cancer therapy
- Others....
Plasma-assisted medical device: surgery

- APC (Argon Plasma Coagulator): ERBE, Germany

Video courtesy of Dr. Jim Barthel
Section Head, Endoscopic Oncology
Medical Director, Endoscopic Oncology Area
Moffitt Cancer Center & Research Institute
Tampa, Florida


Tissue ablation and coagulation

(http://www.erbe-med.de/)
Plasma medicine: where are we heading?

- Decontamination of microorganisms (virus, bacteria, fungi, yeast)

  - E. coli on culture plate
  - In vivo sterilization


- Dental: root canal treatment


- Cancer treatment

  - 6 min x 5 days
  - control

Recent results show exciting effectiveness of plasma in shrinking tumors *in vivo*.


**FE-DBD plasma in air**
...and *in vitro* (melanoma cells)

**Non-thermal Plasma Induces Apoptosis in Melanoma Cells via Production of Intracellular Reactive Oxygen Species**

Sensenig et al., Oct. 29, 2010
Plasmas in ambient air at room temperature

- Voltage: 10-20 kVpkpk
- Frequency: 1-10 kHz
- Power: ~1W
- Distance to finger: 1-3 mm
- Gas: Static air in California

Plasma-biomaterial interactions: surface-active agents?
Plasma-biomaterial interaction: possible agents

<table>
<thead>
<tr>
<th>Possible Agents</th>
<th>Kinetic Energy</th>
<th>Chemical Reaction</th>
<th>Electrostatic</th>
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</thead>
<tbody>
<tr>
<td>Electrons</td>
<td>1-10 eV</td>
<td>ROS/RNS</td>
<td>negative</td>
</tr>
<tr>
<td>Ions</td>
<td>~ 1 eV</td>
<td></td>
<td>positive/negative</td>
</tr>
<tr>
<td>Radicals</td>
<td>4-12 eV (UVC)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Photons</td>
<td></td>
<td>ROS/RNS</td>
<td>10^6-10^7 V/m</td>
</tr>
<tr>
<td>Electric Field</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Effects**
- Bond breaking (e.g., DNA)
- Sputtering
- Oxidation (e.g., O, OH)
- Immune system (e.g., NO)
- Membrane disruption (~10^9 V/m)
- Stimulation
The field of antioxidants and free radicals is often perceived as focusing around the use of antioxidant supplements to prevent human disease. In fact, antioxidants/free radicals permeate the whole of life, creating the field of redox biology. Free radicals are not all bad, nor antioxidants all good. Life is a balance between the two: antioxidants serve to keep down the levels of free radicals, permitting them to perform useful biological functions without too much damage.

### Reactive oxygen species: ROS

<table>
<thead>
<tr>
<th>Radicals</th>
<th>Nonradicals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ROS</strong></td>
<td><strong>ROS</strong></td>
</tr>
<tr>
<td>Superoxide, $\text{O}_2^\cdot$</td>
<td>H$_2$O$_2$</td>
</tr>
<tr>
<td>Hydroxyl, OH$^\cdot$</td>
<td>Hypobromous acid, HOBr$^a$</td>
</tr>
<tr>
<td>Hydroperoxyl, HO$_2^\cdot$</td>
<td>Hypochlorous acid, HOCl$^b$</td>
</tr>
<tr>
<td>(protonated superoxide)</td>
<td>Ozone, O$_3^c$</td>
</tr>
<tr>
<td>Carbonate, CO$_3^\cdot$</td>
<td>Singlet oxygen ($\text{O}_2^1\Sigma_g^+$)</td>
</tr>
<tr>
<td>Peroxyl, RO$_2^\cdot$</td>
<td>Organic peroxides, ROOH</td>
</tr>
<tr>
<td>Alkoxyl, RO$^\cdot$</td>
<td>Peroxynitrite, ONOO$^-$</td>
</tr>
<tr>
<td>Carbon dioxide radical,</td>
<td>Peroxynitrate, O$_2$NOO$^-$</td>
</tr>
<tr>
<td>$\text{CO}_2^\cdot$</td>
<td>Peroxynitrous acid, ONOOH$^d$</td>
</tr>
<tr>
<td>Singlet $\text{O}_2^1\Sigma_g^+$</td>
<td>Peroxomonocarbonate,</td>
</tr>
<tr>
<td></td>
<td>HOOC$\text{O}_2^-$</td>
</tr>
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</table>
## Reactive chlorine/bromine species

<table>
<thead>
<tr>
<th>Radicals</th>
<th>Nonradicals</th>
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<tbody>
<tr>
<td><strong>Reactive chlorine</strong></td>
<td><strong>Reactive chlorine</strong></td>
</tr>
<tr>
<td>Atomic chlorine, $\text{Cl}^*$</td>
<td>Hypochlorous acid, $\text{HOCl}^b$</td>
</tr>
<tr>
<td></td>
<td>Nitryl chloride, $\text{NO}_2\text{Cl}^e$</td>
</tr>
<tr>
<td></td>
<td>Chloramines</td>
</tr>
<tr>
<td></td>
<td>Chlorine gas ($\text{Cl}_2$)</td>
</tr>
<tr>
<td></td>
<td>Bromine chloride ($\text{BrCl}^a$)</td>
</tr>
<tr>
<td></td>
<td>Chlorine dioxide ($\text{ClO}_2$)</td>
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<tr>
<td><strong>Reactive bromine</strong></td>
<td><strong>Reactive bromine</strong></td>
</tr>
<tr>
<td>Atomic bromine, $\text{Br}^*$</td>
<td>Hypobromous acid ($\text{HOBr}$)</td>
</tr>
<tr>
<td></td>
<td>Bromine gas ($\text{Br}_2$)</td>
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<tr>
<td></td>
<td>Bromine chloride ($\text{BrCl}^a$)</td>
</tr>
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</table>

Halliwell, 2006
## Reactive nitrogen species: RNS

<table>
<thead>
<tr>
<th>Radicals</th>
<th>Nonradicals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reactive nitrogen</strong></td>
<td><strong>Reactive nitrogen</strong></td>
</tr>
<tr>
<td>Nitric oxide, NO$^\cdot$</td>
<td>Nitrous acid, HNO$_2$</td>
</tr>
<tr>
<td>Nitrogen dioxide, NO$_2^\cdot$</td>
<td>Nitrosyl cation, NO$^+$</td>
</tr>
<tr>
<td>Nitrate radical, NO$_3^\cdot$</td>
<td>Nitroxyl anion, NO$^-$</td>
</tr>
<tr>
<td>Dinitrogen tetroxide, N$_2$O$_4$</td>
<td>Dinitrogen trioxide, N$_2$O$_3$</td>
</tr>
<tr>
<td>Peroxynitrite, ONOO$^-d$</td>
<td>Peroxynitrate, O$_2$NOO$^-d$</td>
</tr>
<tr>
<td>Peroxynitrous acid, ONOOH$^d$</td>
<td>Peroxynitrous acid, ONOOH$^d$</td>
</tr>
<tr>
<td>Nitronium cation, NO$_2^+$</td>
<td>Alkyl peroxynitrites, ROONO</td>
</tr>
<tr>
<td>Alkyl peroxynitrites, ROONO</td>
<td>Alkyl peroxynitrates, RO$_2$ONO</td>
</tr>
<tr>
<td>Nitryl chloride, NO$_2$Cl</td>
<td>Peroxyacetyl nitrate, CH$_3$C(O)OONO$_2$</td>
</tr>
</tbody>
</table>

Halliwell, 2006
Redox-Directed Cancer Therapeutics: Molecular Mechanisms and Opportunities

ANTIOXIDANTS & REDOX SIGNALING
Volume 11, Number 12, 2009
© Mary Ann Liebert, Inc.
DOI: 10.1089/ars.2009.2541

Georg T. Wondrak

Targeting cancer cell redox homeostasis by small molecule prooxidant intervention

healthy tissue
tumor tissue

ROS
POX

cell death
cell cycle arrest
functional impairment
Figure 6 | Relationship between the levels of ROS and cancer. The effect of reactive oxygen species (ROS) on cell fate depends on the level at which ROS are present. Low levels of ROS (yellow) provide a beneficial effect, supporting cell proliferation and survival pathways. However, once levels of ROS become excessively high (purple), they cause detrimental oxidative stress that can lead to cell death. To counter such oxidative stress, a cell uses antioxidants that prevent ROS from accumulating at high levels. In a cancer cell, aberrant metabolism and protein translation generate abnormally high levels of ROS. Through additional mutations and adaptations, a cancer cell exerts tight regulation of ROS and antioxidants in such a way that the cell survives and the levels of ROS are reduced to moderate levels (blue). This extraordinary control of ROS and the mechanisms designed to counter it allow the cancer cell to avoid the detrimental effects of high levels of ROS, but also increase the chance that the cell will experience additional ROS-mediated mutagenic events and stress responses that promote tumorigenesis. Figure inspired by discussions with Navdeep Chandel, Northwestern University, Chicago, USA.
Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach?

Dunyaporn Trachootham*,†, Jerome Alexandre§ and Peng Huang*

Abstract | Increased generation of reactive oxygen species (ROS) and an altered redox status have long been observed in cancer cells, and recent studies suggest that this biochemical property of cancer cells can be exploited for therapeutic benefits. Cancer cells in advanced stage tumours frequently exhibit multiple genetic alterations and high oxidative stress, suggesting that it might be possible to preferentially eliminate these cells by pharmacological ROS insults. However, the upregulation of antioxidant capacity in adaptation to intrinsic oxidative stress in cancer cells can confer drug resistance. Abrogation of such drug-resistant mechanisms by redox modulation could have significant therapeutic implications. We argue that modulating the unique redox regulatory mechanisms of cancer cells might be an effective strategy to eliminate these cells.
Summary of ROS/RNS Importance in Biological Function

- ROS/RNS are known to play key roles in animal innate immune system and inflammation; plants have an analogous system to protect themselves from microbes.

- ROS/RNS are also known to play key roles in cell signaling processes and other important biochemical functions.

- However, it is well established that excessive ROS/RNS can be carcinogenic and are associated with many degenerative diseases.
Summary of ROS/RNS Importance in Established Therapies

- The mechanisms of antibiotics (e.g. Collins et al., 2007); and at least some antifungal and antiparasitic drugs (e.g. Artemisinin) are thought to involve ROS generation (although mechanisms appear to remain controversial)

- Many cancer therapies are based on the direct or indirect creation of ROS/RNS. Radiation therapy, photodynamic therapy (PDT) and certain chemotherapies all exploit this effect.
Concluding remarks: Ambient gas plasma in healthcare

- Low temperature plasmas create ROS/RNS and other reactive species in relatively high densities at ambient gas temperature

- Preliminary positive results for infection control (disinfection/sterilization and antisepsis); wound healing; cancer therapy; various dermatology applications; dental wound/cavity/biofilm treatment; others

- Results to date promising for future plasma-based healthcare technologies/therapies, perhaps extending beyond ROS/RNS to other reactive/radical species
What’s Next?

• Relation between device operation/design and chemistry not well known; e.g. rare gas jet vs. air plasma? Direct vs. indirect?
• Ions vs. neutrals? Photons and synergies?
• Toxicity issues: what are consequences of long-term biomedical exposure to plasma species?
• How to apply LTP devices to HAI challenges most effectively?
• Medical device approvals: FDA in US; can be slow
• Long term biochemical studies needed for RNS/ROS effects in signaling pathways; genetic responses
• Many others…
<table>
<thead>
<tr>
<th>ROS-modulating agents</th>
<th>Mechanism of action</th>
<th>Current status*</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motexafin gadolinium (gadolinium texaphyrin)</td>
<td>* Pro-oxidant catalyst that induces intracellular superoxide formation and inhibits TrxR, and preferentially accumulates in tumour cells</td>
<td>* Phase III studies in combination with radiation therapy in brain metastases and chemotherapy in haematological malignancies</td>
<td>106</td>
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<tr>
<td>β-Lapachone (ARQ 501)</td>
<td>* Undergoes futile redox cycles catalysed by intracellular NQO1</td>
<td>* Phase I/II studies in tumours overexpressing NQO1</td>
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<td><strong>Inhibitors of the antioxidant system</strong></td>
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<tr>
<td>Buthionine sulfoximine</td>
<td>* Inhibits GSH synthesis</td>
<td>* Phases I/II studies in combination with As$_2$O$_3$ or melphalan</td>
<td>159</td>
</tr>
<tr>
<td>Imexon</td>
<td>* Depletes the GSH pool by binding to thiols</td>
<td>* Phase I/II studies as single agent or in combination with docetaxel and gemcitabine</td>
<td>160</td>
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<tr>
<td>Phenylethyl isothiocyanate</td>
<td>* Conjugates and exports GSH outside cancer cells. * Inhibits GPx and NF-κB</td>
<td>* Preclinical studies in haematological malignancies</td>
<td>84</td>
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<tr>
<td>Mangafodipir</td>
<td>* SOD, catalase and GSH reductase mimetic — increases H$_2$O$_2$ levels in cancer cells but acts as antioxidant in normal cells</td>
<td>* Phase II studies in combination with chemotherapy in liver cancer</td>
<td>161</td>
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<tr>
<td>2-methoxyestradiol</td>
<td>* Inhibits SOD leading to superoxide accumulation</td>
<td>* Phase II studies in prostate, ovary, brain and renal tumours</td>
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<tr>
<td>Tetrathiomolybdate (ATN-224)</td>
<td>* Inhibits cytosolic SOD1</td>
<td>* Phase II studies in melanoma, myeloma, prostate and breast carcinoma</td>
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<tr>
<td><strong>Multiple mechanisms of action</strong></td>
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<td></td>
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<tr>
<td>As$_2$O$_3$</td>
<td>* Inhibits GPx and TrxR * Inhibits the mitochondrial respiratory chain</td>
<td>* Approved for the treatment of relapsing acute promyelocytic leukaemia</td>
<td>103, 163</td>
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<tr>
<td><strong>Unknown mechanism of action</strong></td>
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<td></td>
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<tr>
<td>Elecsclomol (STA-4783)</td>
<td>* Induces rapid ROS accumulation in cancer cells leading to apoptosis * Enhances paclitaxel activity</td>
<td>* Preliminary evidence of activity in combination with paclitaxel in metastatic melanoma * Phase III study suspended</td>
<td>113, 114</td>
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</table>
Low temperature atmospheric pressure plasma sources for microbial decontamination


J Ehlbeck¹, U Schnabel¹, M Polak², J Winter³, Th von Woedtke¹, R Brandenburg¹, T von dem Hagen³ and K-D Weltmann¹

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² Vanguard AG, Friedrichstrasse 78, 10117 Berlin, Germany
³ Webeco GmbH & Co. KG, An der Trave 14, 23923 Selmsdorf, Germany

Table 3. Assortment of recent inactivation results achieved with different APPS. In addition, appendant experimental conditions are given.

<table>
<thead>
<tr>
<th>Discharge type</th>
<th>RF</th>
<th>Expos. time (s)</th>
<th>Gas</th>
<th>MO</th>
<th>(N_0) (cfu)</th>
<th>MO environment</th>
<th>Lit.</th>
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<td>300</td>
<td>(O_3)</td>
<td>Norwalk Virus</td>
<td>—</td>
<td>Liquid</td>
<td>Shin and Sobsey (2003)</td>
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<td>(O_3)</td>
<td>Poliovirus</td>
<td>—</td>
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<td>(O_3)</td>
<td>Coliphage MS2</td>
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<td>1200</td>
<td>air</td>
<td>(E. coli)</td>
<td>(1 \times 10^5)</td>
<td>u</td>
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<td>1800</td>
<td>air</td>
<td>(C. albicans)</td>
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<td>(S. epidermidis)</td>
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<td>u</td>
<td>Bussiahn et al (2010)</td>
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<tr>
<td></td>
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<td>160</td>
<td>Ar</td>
<td>(E. coli)</td>
<td>u</td>
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<td>Time (s)</td>
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</table>
Fluid model: neutral gas flow

Mole fraction of air (log scale)

Gas temperature

[Image of plots showing mole fraction of air and gas temperature]
Fluid model: phase-averaged species density
Fluid model: emission intensity

Mole fraction of air (log scale)

Predicted emission pattern

dark ← bright

Y. Sakyiama et al., Plasma Sources Sci. Technol. 18 (2009) 025022
Infectious Disease Control: Context of Emerging Antimicrobial Resistance (WHO, 2002)

- Since 1940’s, antibiotics substantially reduced threat of infectious diseases.

- They have also contributed to the major gains in life expectancy experienced during the latter part of the 20th century.
• This progress is threatened by emergence and spread of microbes that are resistant to cheap and effective first-choice, or "first-line" drugs.

• Microbial resistance is most evident for: diarrheal diseases, respiratory tract infections, meningitis, sexually transmitted infections, and hospital-acquired infections.
Infectious Disease Control: Context of Emerging Antimicrobial Resistance (WHO, 2002)

• Resistance to first-line antimicrobials requires treatment with second- or third-line drugs: much more expensive and sometimes more toxic as well

• e.g., drugs needed to treat multidrug-resistant forms of tuberculosis are over 100 times more expensive than the first-line drugs used to treat non-resistant forms.
When a theory of aging ages badly

Jérôme Lapointe · Siegfried Hekimi

Core Statement

Production of mitochondrial ROS is THE cause of aging.

Associated Hypotheses
- Mitochondrial oxidative damage accumulates with chronological age.
- Mitochondrial function declines with chronological age.
- Mitochondrial ROS production increases with chronological age.
- Global oxidative damage to proteins, DNA and lipids increases with chronological age.
- Oxidative damage participates in the functional deterioration of aging.

TEST

<table>
<thead>
<tr>
<th>Decreasing ROS levels with dietary antioxidants or by genetic over-expression of antioxidant activities</th>
<th>Increasing ROS levels by genetic inactivation of antioxidant activities</th>
<th>Determining oxidative status in long-lived species and mutants.</th>
<th>Analyzing mitochondrial function and oxidative biomarkers throughout life and their link to disease, including in long-lived mutants</th>
</tr>
</thead>
</table>

Expected
- Long lifespan
- Short lifespan
- Decreased ROS production. Less oxidative damage.

Observed
- Normal lifespan
- Detrimental effects
- Normal or long lifespan
- Normal or more oxidative damage

Falsified

Verified

Fig. 1 Schematic representation of the core statement and the associated hypotheses of the MFRTA, which have been tested by multiple studies in mammals. Taken together, the findings of these analyses have led to the conclusions that the core statement of the theory can now be considered as falsified in contrast to the associated hypotheses that have been verified by a variety of measures.
Plasma-cell/tissue interaction for antisepsis and wound healing

prokaryotes (bacteria)
- Gram positive: e.g. B. subtilis (vegetative cell or spore)
- Gram negative: e.g. E. coli

eukaryotes (animal or plant)

thin film on agar
planktonic
biofilm
in vivo
Plasma-induced wound healing: cell growth

3T3 mouse fibroblasts (30s treatment)

Initial concentration of cells (log-scale)

stimulates cell growth rate
needs careful control of dose and power

Human skin fibroblasts

with plasma
control

R.S. Tipa et al., ICPM-2 (2009)
(Courtesy of R.S. Tipa)

T. Nosenko et al., ICPM-2 (2009)
(Courtesy of T. Nosenko)
Plasma-induced wound healing: various anecdotal reports

Treatment of Topical Wounds: Tissue Regeneration: Suppurated Burn Wound (2009 conference)*

*Richard M. Satava, MD FACS
Professor of Surgery
University of Washington
*Concluding Remarks*

- Infectious disease remains a major worldwide threat and various factors suggest it will grow with time

- Hospital-acquired infections are particularly dangerous with rise in antimicrobial resistance and few new antimicrobials are being developed

- Suggest need for new field of infection control science and engineering – including plasma-assisted technologies, perhaps analogous to other plasma-related fields such as semiconductor processing that have used plasma–assisted technologies