Low Temperature Plasmas for Biomedical Applications

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Low Temperature Plasmas “In the Kitchen” * Cal

* Plasma 2010
Plasma Healthcare

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

- APC (Argon Plasma Coagulator): ERBE, Germany
  (http://www.erbe-med.de/)


*Tissue ablation and coagulation*
APC (Argon Plasma Coagulator)
Example in *Gastrointestinal Oncology*

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

0-15 s: visualization and targeting of area of Barrett’s precancerous epithelium in the esophagus (dark pink area).

15 s – 1.5 min: pulsed (16 Hz; 12 W; Ar 1.2 l/m). Surface coagulation from thermal effect is obvious.

1.5 – 2 min: physical removal of coagulum to expose underlying tissue layer.

2 min – end: pulsed (16 Hz; 6 W; Ar flow at 1.2 l/m) Non-thermal effects may be predominating. Streamer formation to treatment surface is much less frequent.

*What atoms and uncharged molecular entities are being delivered to the tissue surface?*
Plasma-assisted surgical device

- Coblation (Cold + Ablation) by Arthrocare, CA USA
  (http://www.arthrocare.com/)

K. Stalder et al.,
Coblation®: Tonsillectomy and Prostate Resection

FIG. 2. Flow of current during prostate Coblation.
The PEAK PlasmaBlade™ tissue dissection devices are a family of new surgical instruments that use PEAK Surgical’s proprietary Pulsed Plasma Technology™ for soft-tissue cutting and coagulation with minimal thermal injury. These PlasmaBlades work in conjunction with the PULSAR™ Generator to create the PEAK Surgery System (Fig 1).
Plasma-assisted surgical device -3

- PEAK (Plasma Electron Avalanche Knife):
  by D.V. Palanker (originally Stanford)


Major Thrust for Plasma Medicine: Infection Control

- Global factors promoting spread of infectious disease
- Hospital-acquired infections
- Nature of human-microbe relationship
- Growing challenge of antimicrobial resistance
- Immune system and role of RNS/ROS
- Ambient gas (air) plasmas: mimicking innate immune system chemistry
**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
- global climate change disrupting ecosystems
- antimicrobial resistance: inexorable rise in number of resistant microbes limits use of traditional infection control (e.g. antibiotics)
- few new antimicrobial drugs in pipeline
- emerging threat of bioterrorism
Major Problem: Hospital-Acquired Infections: HAI, or ‘Nosocomial’ Infections

2002 in US: 1.7 million HAI s & 99,000 deaths

*Hand hygiene.* Health care workers must wash/disinfect hands many times per day to prevent infection transmission of (often multi-drug resistant) bacteria. Poor compliance overall.

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

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

*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.

*Prion and biomolecule deactivation*. Transmissible spongiform encephalopathies (TSEs) are neurogenerative diseases that are thought to be caused by mis-folded proteins (‘prions’). Challenge is to disinfect heat- and alkaline-sensitive, expensive surgical and diagnostic equipment.
Us vs. Them: Not a Fair Fight!

<table>
<thead>
<tr>
<th>Variable</th>
<th>Microbes</th>
<th>Humans</th>
<th>Factor</th>
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</thead>
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<tr>
<td>No. on earth</td>
<td>$5 \times 10^{31}$</td>
<td>$6 \times 10^9$</td>
<td>$\sim 10^{22}$</td>
</tr>
<tr>
<td>Mass, metric tons</td>
<td>$5 \times 10^{16}$</td>
<td>$3 \times 10^8$</td>
<td>$\sim 10^8$</td>
</tr>
<tr>
<td>Generation time</td>
<td>30 min</td>
<td>30 years</td>
<td>$\sim 5 \times 10^5$</td>
</tr>
<tr>
<td>Time on earth, years</td>
<td>$3.5 \times 10^9$</td>
<td>$4 \times 10^6$</td>
<td>$\sim 10^3$</td>
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</table>

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. the 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.

- In some diseases, resistance is developing for virtually all currently available drugs: a post-antibiotic era may be coming!
Why Is IDSA Concerned?
Resistant Bacterial Strains Spread Rapidly

(Infectious Disease Society of America: IDSA)
Total Approved Antibacterials: US

Pharmaceutical companies not developing new drugs!

Spellberg, et. al., C/D May 1 2004, Modified
Dr. Richard Whitley, MD, FIDSA; President, IDSA*

“Without effective antimicrobial drugs, modern medical treatments such as operations, transplants, intensive care, cancer treatment and care of premature babies will become very risky if not impossible.”

“People have this crazy belief that hospital acquired infections are the result of sloppy medicine. Not so. They are the result of very sick people with tremendously sophisticated levels of intensive medical care being delivered in a concentrated environment (i.e., a hospital). Crowd a bunch of sick people together with plastic catheters, mechanical ventilators, and nasty bacteria, and such infections are inevitable. What we are learning is that we have to go above and beyond normal to stop these infections from happening. Research is needed on how best to do this. It's not as simple as people think. You can't stop the spread of the (microbial) resistance itself. It is inevitable.”

•Author: Rising Plague: The Global Threat from Deadly Bacteria and Our Dwindling Arsenal to Fight Them
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
- cannot discriminate between host and invader
- acquired immune system: *exquisitely selective*
- chronic inflammation disease widespread, especially for aging populations, and may be result of innate system malfunction as bodies age

Note: *ROS and RNS* exposures must be short-term to avoid well-known health/disease problems with ‘free radicals’
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>
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<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>
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<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>
<td></td>
</tr>
</tbody>
</table>

ROS/RNS utilized in innate immune system response

Figure 3-12
Kuby IMMUNOLOGY, Sixth Edition
© 2007 W.H. Freeman and Company
Antimicrobial species generated from oxygen and nitrogen

Reactive oxygen species (ROS)
- $\cdot O_2^-$ (superoxide anion)
- OH$^-$ (hydroxyl radical)
- $H_2O_2$ (hydrogen peroxide)
- ClO$^-$ (hypochlorite anion)

NADPH
phagosome oxidase

Superoxide dismutase

$O_2$ Oxygen

$\cdot O_2^-$ Superoxide anion

$H_2O_2$ Hydrogen peroxide

Myeloperoxidase

Cl$^-$ Chloride ion

HCIO$^-$ Hypochlorite

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

NO Nitric oxide

ONOO$^-$ Peroxynitrite

$NO_2$ Nitrogen dioxide

Figure 3-13
Kuby IMMUNOLOGY, Sixth Edition
© 2007 W. H. Freeman and Company
Microbial targets of ROS/RNS

Fang, Nature Reviews Microbiology, 2004
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
Implications for Plasma Technology vs. Infections

1. The challenge is huge. Antibiotic resistance and hospital-acquired infections, coupled with other infectious disease problems worldwide: infection control is imperative!

2. ROS and RNS are central players in the body’s defense against ID agents as well as playing other key biochemical roles.

3. Atmospheric pressure, low temperature plasmas are prolific, inexpensive, and simple generators of ROS and RNS.

The opportunity is clear for plasma-assisted infection control: Chemistry that mimics the innate immune system
Plasma sources: direct and remote exposure

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

- remote exposure
  - reactive neutrals (long-lived)
  - UV photons

Comparison of Direct and Indirect Effects of Non-Thermal Atmospheric-Pressure Plasma on Bacteria

Gregory Fridman,* Ari D. Brooks, Manjula Balasubramanian, Alexander Fridman, Alexander Gutsol, Victor N. Vasilets, Halim Ayan, Gary Friedman
MPI Bacterial sterilization – E-coli results*

* HandPlaSter, Courtesy Prof. Dr. G. Morfill, Max Planck Inst. Extraterrestrial Physics
MPI Bacterial sterilisation - Enterococcus results

$10^4$ dilution

V = 18 kVpp
distance: 6 mm

Enterococcus (g +ve)

$bacteria\ number$ vs $treatment\ time\ (s)$

$10^4$ dilution

Original level
Plasma-induced wound healing: cell growth

stimulates **cell growth rate**
needs careful control of dose and power

**3T3 mouse fibroblasts (30s treatment)**

- With plasma
- Control

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

**Human skin fibroblasts**

- Control
- 5 min irradiation
- 10 min irradiation

T. Nosenko et al., ICPM-2 (2009)
(Courtesy of T. Nosenko)
Gas plasma treatment at atmospheric pressure

localized cell treatment

dental treatment

http://medicalphysicsweb.org/

The Plasma Needle

- non-thermal
- RF (13.56MHz) driven
- atmospheric pressure
- millimeter/micrometer size

E. Stoffels et al,
Plasma needle: ring-shaped killing pattern!? 


- flow velocity: 0.5 ~ 4 m/s
- gap distance: 2.5 ~ 4 mm
- target: *S. mutans*

Discharge image

Light emission (side view)

Killing pattern (top view)

(Images courtesy of Prof. John Goree)
Plasma needle: coupled simulation model

Neutral Gas flow

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

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

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

Plasma dynamics

\[ \frac{\partial n_i}{\partial t} + \nabla \cdot \Gamma_i = S_i \quad \text{(mass conservation)} \]

\[ \Gamma_i = \text{sgn}(q_i) n_i \mu_i \mathbf{E} - D_i \nabla n_i + n_i \mathbf{u} \quad \text{(drift-diffusion)} \]

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

\[ \varepsilon_0 \nabla \cdot \mathbf{E} = \sum q_i n_i \quad \text{(Poisson’s equation)} \]
Plasma Domain and Mesh (Early Studies)

Mesh size: 3 ~ 140 μm
Number of mesh: 3,000 ~ 5,000
Shape function: Lagrange-quadratic
Number of DOF: 40,000 ~ 70,000

**RF (13.56 MHz)**

**Needle**

**Planar electrode**

**Axis of symmetry**

ϕ = 30 μm

1 mm
Plasma needle: reproduced emission pattern

Predicted flow field

He rich ← N₂ rich

Predicted emission pattern
dark ← bright

Y. Sakyiama et al., Plasma...
Plasma needle: one-way coupled plasma model for detailed chemistry

Predicted flow field

He rich → N₂ rich

insulator

needle

S. mutans

on-axis (He rich)

off-axis (Air rich)

S. mutans

1D plasma model

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

\[
\Gamma_i = \text{sgn}(q_i) n_i \mu_i E - D_i \nabla n_i
\]

\[
\frac{\partial (n \varepsilon)}{\partial t} + \nabla \left( \frac{5}{3} \varepsilon \Gamma_e - \frac{5}{3} n_e D_e \nabla \varepsilon \right) = -\Gamma_e \cdot E - Q_{\text{inel}} - Q_e
\]

\[
\varepsilon_0 \nabla \cdot E = e \sum_i q_i n_i
\]
Plasma needle: plasma chemistry model

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
Plasma needle: charged particle density

- **On-axis (no inactivation)**
  - $\text{He}_2^+$
  - $\text{O}^+$
  - $\text{H}_2\text{O}^+, \text{H}^+, \text{OH}^+$
  - $\text{N}^+, \text{N}_2^+$
  - $\text{NO}^+$

- **Off-axis (inactivation)**
  - $\text{He}_2^+$
  - $\text{N}^+, \text{N}_2^+$
  - $\text{NO}^+$
  - $\text{O}^+, \text{O}_2^+$

The graphs show the density variation with respect to radial distance ($r$) in millimeters. The density is given in units of $\text{m}^{-3}$. The left graph represents the on-axis condition without inactivation, while the right graph shows the off-axis condition with inactivation.
Plasma needle: neutral species density

On-axis (no inactivation)

- N, N⁺, N₂⁺
- O, O⁺, O₂⁺
- H, OH, H₂

Off-axis (inactivation)

- He⁺, He₂⁺
- O, O⁺, O₂⁺
- N, N⁺, N₂⁺
- H, OH, H₂
- NO

Graphs showing the density of different species as a function of radial position (r in mm).
Plasma needle: flux onto treated surface

On-axis (1mm gap)

Off-axis (2mm gap)

S. mutans
Plasma needle: TALIF measurement

(collaboration with Dr. Schulz-von der Gathen at Bochum, Germany)

TALIF setup at Bochum

- flow rate: 1 slpm
  (~1.3 m/s at the needle tip)
- gap distance: 3 mm
- consumed power: ~3 W

Plasma needle: ground state atomic oxygen

(collaboration with Dr. Schulz-von der Gathen at Bochum, Germany)
Plasma bullet
In partial collaboration with
Prof. Mounir Laroussi
(Old Dominion Univ.)
Plasma bullet: one-way coupled model (neutral flow)

Mole fraction of air

Governing equations

\[ \nabla \cdot (\rho \mathbf{u}) = 0 \]

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

\[ \nabla \cdot (\rho \mathbf{u} u_i) = -\nabla p - \nabla \cdot \mathbf{\tau} \]
Plasma bullet: one-way coupled model (plasma)

Mole fraction of air

Assumption

\[ E_z = 2 \times 10^5 \text{ V/m} \]

100 ns

100 μs (10 kHz)

\[ |E| = \sqrt{|E_r|^2 + |E_z|^2} \]
Plasma bullet: ring-shaped emission

Light emission intensity (at 20 mm)

Plasma bullet: collapse of ring-shaped pattern

Light emission intensity at 20 and 40 mm

Surface air DBD

In partial collaboration with

Prof. Greg Morfill
(Max Planck Inst., Garching, Germany)
Surface air DBD: motivation

Discharge image (top view)

Inactivation of *E. coli*

Device setup (side view)

- ground electrode (~φ 1 mm)
- micro-discharge
- insulator
- powered electrode
- 5kV, 10kHz

control

15s exposure

(Photos courtesy of Prof. G. Morfill)
Surface air DBD: description of 0-D model

\[ P = \frac{V}{T_{rep}} \int_0^{\tau_e} e \Gamma_e \cdot \mathbf{E} \, dt = \frac{\tau_e}{T_{rep}} e \mu_e n_e E^2 V \]

\[ J = e \mu_e n_e E \]

\[ n_e = \frac{J^2 V \tau_e}{e \mu_e T_{rep} P}, \quad E = \frac{P T_{rep}}{J V \tau_e} \]

\[
\frac{\partial n_i}{\partial t} = \sum_i S_{ij} - \frac{1}{4} \gamma_i n_i v_i \frac{A}{V}
\]

n_e, E (or \( \varepsilon \))
Surface air DBD: plasma chemistry in humid air

48 species

- 11 negative particles: $e, O^-, O_2^-, O_3^-, O_4^-, H^-, OH^-, NO^-, N_2O^-, NO_2^-, NO_3^-$
- 16 positive particles: $N^+, N_2^+, N_3^+, N_4^+, O^+, O_2^+, O_4^+, NO^+, N_2O^+, NO_2^+, H^+, H_2^+, H_3^+, OH^+, H_2O^+, H_3O^+$
- 21 neutrals/radicals: $N, N^*, N_2, N_2^*, N_2^{**}, O, O^*, O_2, O_2^*, O_3, NO, N_2O, NO_2, NO_3, N_2O_5, H, H_2, OH, H_2O, HO_2, H_2O_2$

630 reactions

- 21 electron impact excitation/ionization/dissociation
- 76 electron recombination/attachment
- 159 charge transfer
- 245 ion recombination
- 129 neutral-neutral reactions
Surface air DBD: ozone may be key ROS
Surface air DBD: flow tube reactor simulation

0-D plasma chemistry
\[
\frac{\partial n_i}{\partial t} = \sum_j S_{ij} - \frac{1}{4} r_i n_i \nu_i \frac{A}{V}
\]
(48 species, 630 reactions)

2-D reactive flow
- O + O_2 + M → O_3 + M
- O_3 + NO → NO_2 + O_2
- O + NO_2 → NO + O_2

NO distribution
(color: linear scale, contour: log scale)

O_3 distribution
(color: linear scale, contour: log scale)
Recent measurements: $O_3$ in surface DBD


- Power: 0.01-0.1 W/cm$^2$
- Voltage: $\sim$10 kV$_{pk}$
- Frequency: $\sim$5 kHz

Simulation results: $12\times10^{15}$ cm$^3$
Concluding Remarks

- Low temperature plasmas appear poised to have significant impact in various healthcare-related applications
- Medical applications still limited for direct plasma-tissue/cell interaction applications (antisepsis/wound healing/cancer cell apoptosis)
- Infection control via air/rare gas LTP looks especially promising
- One area of interest in our group: applications in developing world – all HAI and ID problems amplified in low- and middle-income countries; LTP device strengths include low cost and simplicity: hand-held and rechargeable battery powered?
Concluding Remarks: What’s Next?

- Relation between device operation/design and chemistry not well known; e.g. rare gas jet vs. air plasma?
- Plasma-liquid interactions promising (not presented here)
- 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 repsonses
- Many others…