Plasma Infection Control: Facing a Grand Challenge

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Plasma Healthcare

- Surgery; cell/tissue removal, coagulation

- Wound healing, blood clotting

- Cancer therapy

- Infection control: sterilization, disinfection, antisepsis, *in-vivo* anti-microbial action

- Others....
Plasma sources: direct and remote exposure

- charged particles
- electric field/charge accumulation
- reactive neutrals
- 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
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

Present and future grand challenge of infection control science and engineering
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
Global examples of emerging, re-emerging and deliberately introduced diseases

(Morens et al., Nature, 2004)

Infectious Disease

<table>
<thead>
<tr>
<th>Infectious diseases</th>
<th>Annual deaths (million)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory infections</td>
<td>3.96</td>
</tr>
<tr>
<td>HIV/AIDS</td>
<td>2.77</td>
</tr>
<tr>
<td>Diarrhoeal diseases</td>
<td>1.60</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>1.56</td>
</tr>
<tr>
<td>Vaccine-preventable childhood diseases</td>
<td>1.12</td>
</tr>
<tr>
<td>Malaria</td>
<td>1.27</td>
</tr>
<tr>
<td>STDs (other than HIV)</td>
<td>0.18</td>
</tr>
<tr>
<td>Meningitis</td>
<td>0.17</td>
</tr>
<tr>
<td>Hepatitis B and C</td>
<td>0.16</td>
</tr>
<tr>
<td>Tropical parasitic diseases</td>
<td>0.13</td>
</tr>
<tr>
<td>Dengue</td>
<td>0.02</td>
</tr>
<tr>
<td>Other infectious diseases</td>
<td>1.76</td>
</tr>
</tbody>
</table>
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.
Major Problem: Hospital-Acquired Infections: HAI, or ‘Nosocomial’ Infections

Prion and biomolecule infections. 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.
Hospital-Acquired Infection: Known Causes and Complicating Factors

**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. (See G. Morfill and HandPlaster)

**Antimicrobial resistance.** Described in greater detail below; major complicating factor in HAI control
Us vs. Them: Not a Fair Fight!

<table>
<thead>
<tr>
<th>Variable</th>
<th>Microbes</th>
<th>Humans</th>
<th>Factor</th>
</tr>
</thead>
<tbody>
<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>
</tr>
</tbody>
</table>

Microbes and Humans Co-Evolved

• Typical human body has $\sim 10^{14}$ microbes (mostly bacteria), several percent or so on the skin; most reside in the gut

• Typical skin bacterial density estimates $\sim 10^3 - 10^8$ cm$^{-2}$

• Gut microbe mass $\sim 1.5$ kg!

• Current thinking suggests *human-microbe symbiosis*

*Humans are walking petri dishes for microbes!*
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.
Infectious Disease Control: Context of Emerging Antimicrobial Resistance (WHO, 2002)

- 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.
In some diseases, resistance is developing for virtually all currently available drugs: *a post-antibiotic era may be coming!*

But modern medicine *relies on invasive procedures*, and antimicrobials are essential to avoid subsequent, often inevitable infections.
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., CID May 1 2004, Modified
“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.”

Dr. Richard Whitley, MD, FIDSA; President, IDSA*

*U.S. and European Experts Applaud Creation of New Transatlantic Task Force on Global Antibiotic Resistance Threat

Press release Nov. 5, 2009, IDSA
How to Fight HAI?

Dr. B. Spellberg, Infectious Disease Society*

• Author: Rising Plague: The Global Threat from Deadly Bacteria and Our Dwindling Arsenal to Fight Them

“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. ...”
Why We Probably Need a New Field of Infection Control Science and Engineering

Dr. B. Spellberg, IDS:

“...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.”
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*
Immune System: Innate and Adaptive

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

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

Time after infection
- Hours: 0, 6, 12
- Days: 1, 3, 5

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, Reactive oxygen and nitrogen species, Antimicrobial peptides</td>
<td>Phagocytosis, Inflammatory mediators, Antigen presentation, Reactive oxygen and nitrogen species, Cytokines, Complement proteins</td>
<td>Antigen presentation, Costimulatory signals, Reactive oxygen species, Interferon, Cytokines</td>
<td>Lysis of viral-infected cells, Interferon, Macrophage activation</td>
</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)**
- \( \cdot O_2^- \) (superoxide anion)
- OH\(^+\) (hydroxyl radical)
- \( H_2O_2 \) (hydrogen peroxide)
- ClO\(^-\) (hypochlorite anion)

\[ \text{O}_2 \xrightarrow{\text{Superoxide dismutase}} \cdot O_2^- \xrightarrow{\text{NADPH phagosome oxidase}} H_2O_2 \xrightarrow{\text{Myeloperoxidase}} \text{HClO}^- \]

**Reactive nitrogen species (RNS)**
- NO (nitric oxide)
- \( NO_2 \) (nitrogen dioxide)
- \( ONOO^- \) (peroxynitrite)

\[ \text{NO} \xrightarrow{\text{Nitric oxide}} \xrightarrow{\text{ONOO}^-} \text{Peroxynitrite} \xrightarrow{\text{Peroxidase}} \text{NO}_2 \]

Figure 3-13
*Kuby IMMUNOLOGY, Sixth Edition*
© 2007 W.H. Freeman and Company
Typical Chemistries Produced by Air Plasmas (T ~ 300K)

Y. Sakiyama

Y. Sakiyama
Typical Chemistries Produced by He/Air Plasmas

Y. Sakiyama

$\text{He}_2^+ + \text{N}^+, \text{N}_2^+ + \text{O}^+, \text{O}_2^+ + \text{NO}^+$

Charged species

![Graph showing density vs. r (mm)]
Typical Chemistries Produced by He/Air Plasmas

Y. Sakiyama

Neutral species

\( \text{He}^+, \text{He}_2^*, \text{N}, \text{N}^*, \text{N}_2^* \), \( \text{O}, \text{O}^*, \text{O}_2^* \), \( \text{H}, \text{OH}, \text{H}_2^* \), \( \text{NO} \)
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
A certain fraction of pathogens, however, may escape this rapid but moderately effective manifestation of “innate immunity” and may generate within a few days a large progeny of pathogens.

The massive production of ROS (oxidative burst) by activated macrophages in the inflammatory environment provides a first line of defense against environmental pathogens.

A certain fraction of pathogens, however, may escape this rapid but moderately effective manifestation of “innate immunity” and may generate within a few days a large progeny of pathogens.
Antigenic peptides generated within the activated macrophages by the breakdown of pathogens are presented by major histocompatibility complex (MHC) determinants to the antigen receptors (AR) of T lymphocytes.

This interaction triggers the proliferation and differentiation of the T cells and leads within a few days to a large progeny of immunological effector cells. The effector cells provide a highly effective and antigen-specific immunological defense.

Without this effect, the T lymphocytes would require relatively large concentrations of antigenic peptides and would lose valuable time in their “race” with the proliferating pathogens. In this situation time may be a matter of life or death for the organism.

ROS that are concomitantly produced by the activated macrophages in the inflammatory environment enhance the AR-mediated signal cascades and decrease thereby the activation threshold of the T cells.

Regulated increase in OS or NO production leads to *temporary* imbalance – basis of redox regulation. *Persistent production of abnormally large amounts of ROS/RNS* may lead to persistent changes in signal transduction and gene expression, leading in turn to pathological conditions.
Implications for Plasma Assisted Infection Control

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

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

- 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 can be used on/near body
*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
*Concluding Remarks*

- Plasma-assisted infection control addresses *environment*, *devices*, and *living tissue*

- In all cases, but especially for living tissue interactions, ROS and RNS created by plasma appear to play important roles

- Analogous chemistry in innate and perhaps acquired immune systems

- Infection control during invasive procedures: plasma-generated ROS/RNS *can be used in/on body*

- Key to avoiding damage from ROS/RNS is to limit duration of exposure
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…