Low-temperature Plasma at Penn State: Effects on Cultured Metastatic Breast Cancer Cells

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Low-temperature plasma group at PSU consists of faculty, a graduate student and undergraduates

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Low-temperature plasma differs significantly from fusion-related plasma

- What is low-temperature plasma (LTP)?
  - Different than the high-temperature plasma that is seen elsewhere, driven by fusion-related research
  - Those plasmas have electrons and ions that are usually in thermal equilibrium with temperatures of millions of degrees
  - *LTP has ions and neutral particles that have low temperatures*... near room temperature in some cases

- Not produced in a vacuum chamber, but often in the ambient atmosphere
  - Chemical environments are more complex due to the many different atomic and/or molecular constituents
The complex environment gives rise to complex chemistry

- Ions and neutral particles (i.e., massive particles) may be at room temperature, but electrons can still be very energetic (~ eV)
- Collisions of these electrons with massive particles leads to a different outcomes
  - Ionization – liberation of an electron
  - Excitation – electronic, vibrational, etc.
  - Dissociation – breaking of molecular bonds
- **Dissociation and excitation can lead to the production of very chemically reactive species**
Enhanced chemistry from reactive species is context dependent

- **Chemical species produced are dependent on constituents of the medium and electron energy distribution**
  - Production in liquids, such as water and saline, will give rise to OH and O$_3$, etc.
  - Production with ambient air will produce NO$_x$, OH, O$_3$, etc. (RONS)
  - Production with a carrier gas (plasma jet) will provide excited species of the carrier gas, He or Ar, as well

- In addition, charged particles and radiation, visible through the UV, are produced
The enhanced chemistry leads to many different potential applications

• Biomedical: a rapidly growing field known as *plasma medicine*
  – Sterilization: destruction of infectious microbes, suspected due to reactive oxygen
  – Cancer treatment: selective induction of apoptosis or necrosis
  – Biofilm destruction: penetrate polysaccharide shell to reach microbes

• Materials: from large surface treatments to nanoparticles
  – Surface functionalization: improve bonding by adding chemical groups to surface
  – Surface energy alteration: hydrophobic vs. hydrophilic surfaces

• Combustion: improvement of combustion
  – Reduction of ignition times: enhanced dissociation prior to spark ignition
  – Reduction of pollutants: enhanced chemistry furthers completion of combustion
The Penn State LTP group is investigating several applications in this growing field

- Our group pursues undergraduate education and training in science and engineering with focus on low-temperature plasma
  - Materials applications: functionalization of plastics and CNTs
  - Aerospace applications: leading-edge plasma discharge
  - Plasma medicine: cancer investigations, among others
- We utilize four primary discharge geometries:
  - Planar dielectric barrier discharge (DBD)
  - Surface DBD
  - Atmospheric-pressure plasma jet (APPJ)
  - Liquid discharge
Plasma generation equipment includes high-voltage amplifiers and a high-voltage pulser

- **Our laboratory utilizes both low-frequency (kHz) alternating voltage systems and high-voltage ns-pulse equipment**
  - Trek 10/40 and Trek 10/10 high-voltage amplifiers output up to 10 kV peak waveforms at up to 20 kHz (1000 × amplification of input waveform)
    - Used for gas discharges
  - PT-55N high-voltage pulser produces pulses at a 1 Hz rate at 10’s of kV (DC charge voltage dependent)
    - Used for liquid discharges
Planar DBD is utilized for materials applications, but will be extended to plasma medicine

- Parallel copper plates (~ cm’s in extent), covered with 1-mm-thick quartz plates
- Driven with a high-voltage amplifier (Trek 10/40) at 2 kHz, 7–10-kV-peak sine wave, ambient air as the working gas
- Exhibits micro-filamentary behavior, though typically with uniform filling of the DBD gap
Leading-edge DBD is utilized for aerospace applications

- Consists of copper tape covered with Kapton tape across the leading edge of an airfoil section
- Driven with Trek 10/40 at 2 kHz with voltages from 7–10 kV peak
- *Discharge is in ambient air across the gap formed by the copper tape*
Large diameter APPJ is also used for materials modification purposes

- 0.25” ID, 0.375” OD plastic tube with a ring ground electrode on the end and a copper rod, 0.09” diameter, as a high-voltage pin electrode
- Driven with Trek 10/40 at 5 kHz with 2 – 4 kV peak voltage, helium carrier gas
- Functionalized polyethylene with carbonyl groups with goal of improved bonding
LTP-induced chemistry is of interest for biomedical applications – plasma medicine

• LTP is compelling for many biomedical applications because it produces “dry” chemistry, but also **minimal or no thermal effects**

• The primary chemical species of interest for biomedical applications are nitric oxide (NO) and the various forms of reactive oxygen species (ROS)
  – Critical for signaling in the human body
  – NO in particular is a very important biomolecule (1998 Nobel Prize in Medicine)
  – Enhances angiogenesis and cell proliferation (Luo, 2005)

• ROS is also important for cellular signaling, but excessive oxidative stress leads to cell death through different mechanisms
  – Of interest for cancer treatment, malignant cells higher metabolic rate may be more susceptible to oxidative stress

Penn State LTP group uses a plasma “syringe” for biomedical investigations

- **Designed to mimic the dimensions of a 16-gauge biopsy needle**
  - 22-gauge syringe needle (high-voltage electrode) through which He flows
  - Polyether ether ketone (PEEK) polymer sleeve acts as dielectric barrier
  - Ring electrode on the tip
  - Driven with Trek 10/40 at 5 kHz and 2–3.5 kV peak voltage waveform
  - Plasma jet extends ~ cm from the end of the PEEK tube
  - Non-thermal nature has been verified
Effect of the plasma syringe on metastatic breast cancer cell cultures is being investigated

- In conjunction with Professor Andrea Mastro, a breast cancer researcher, we have been investigating the effects of LTP on metastatic breast cancer cells
- MDA-MB-231 human metastatic breast cancer cells
- Cells were cultured in 96- or 24-well cell culture plates
  - DMEM, 5% fetal bovine serum, penicillin 100U/ml/streptomycin 100 µg/ml, and non-essential amino acids in a 37 °C, 5% CO₂ humidified incubator
  - Cells tested negative for mycoplasma
- Prior to plasma exposure, cell culture medium was removed and replaced with phosphate buffered-saline (PBS)
  - Quantity of PBS dependent on cell culture well size (96- vs. 24-well plate)
  - Plasma and control (He flow only) were both undertaken
Experiments were undertaken at varying plasma exposure times

- Initial experiments were conducted in 96-well plates with PBS as a buffer
  - Mass flow rate of $\approx 200$ sccm of helium
  - The amount of buffer was varied from 50 $\mu$L to 200 $\mu$L
  - This was done to protect the cells from the kinetic impact of the gas flow
  - Exposure times up to 3 minutes
- *Hypothesis is that reactive species generated from the plasma jet can diffuse through PBS*
Effects have been observed for both control (helium flow only) and plasma discharges

- Cell culture damage was observed with only helium flow, as well as plasma
  - A larger amount was observed for the plasma jet (2–3 × )

50 μL PBs, 3 minutes exposure
Results of initial experiments indicate that kinetic effects of the gas flow cannot be ignored

• Holes in the cell culture were observed even for the control samples, though at a smaller extent
• The size of the 96-well plates was also an issue
  – Helium backflow produce corona plasma around the ring ground electrode
• New experiments were planned using larger cell culture wells (24-well plates)
  – Prevent helium backflow
  – Provide more flexibility for the amount of buffer added
• A modification to the ring ground electrode prevented the jet from reaching as deeply into the wells
  – Required an increase in flow rate (above the resolution of the flow meter) to increase the plasma jet length in order to couple to the PBS surface (1 slm estimated)
Additional experiments identified a buffer volume that reduced kinetic effects

- Subsequent experiments utilized buffer volumes from 100 μL to 350 μL under the same electrical output conditions (5 kHz, 3 kV peak)
- Clear differences between effects on cell cultures were observed
- It is apparent that the plasma is having a measurable effect on the cells beyond the gas flow kinetics
More investigation is required to understand the phenomena at work

• During plasma exposure, what may have been chemiluminescence was observed in the interaction zone with the PBS
  – A chemical interaction of phosphorus with reactive oxygen?
  – Unknown at the present moment
• Measurement of reactive oxygen and nitrogen species (RONS) in the PBS solution after plasma exposure
  – End product assays for hydrogen peroxide and nitrate/nitrite
• Optical emission spectroscopy of species in the plasma jet
• FTIR spectroscopy to identify chemical constituents in the plasma plume/ambient air interaction region
What is next for the Penn State LTP group?

- Continuation of MDA-MB-231 cell line experiments with end-product assays and dead/alive assays
- Improvement of diagnostic capabilities
  - Spatially-resolved optical emission spectroscopy using an Ocean Optics HR2000 with appropriate optics (Wilson project)
  - Schlieren imaging for plasma plume visualization (Brubaker project)
  - Moire deflectometry for electron density and temperature measurement (Brubaker project)
  - UV/VIS absorption spectroscopy and FTIR spectroscopy for characterization of chemical species (use of Materials Characterization Laboratory User Facility)
Schlieren and Moire systems are nearly online

- Imaging of a heated soldering iron are shown
Summary and Conclusions

• The Penn State LTP group is engaged in a wide variety of investigations
  – Materials modifications
  – Aerospace applications
  – Biomedical investigations

• The effect of LTP on MDA-MB-231 human metastatic breast cancer cells is investigated
  – Hypothesis is that reactive chemical species generated by plasma jet diffuse through PBS buffer solution, producing oxidative stress on the cell cultures

• Experiments indicate that the kinetic effects of the gas flow can damage the cultured cells
Summary and Conclusions

- The damage to cell cultures from the plasma jet is more pronounced
  - Perhaps due to diffusion of reactive species
  - Chemiluminescent reaction with phosphorus?
- Quantification of the reactive species produced in PBS is required
- Measurement of species produced by the plasma jet through optical emission spectroscopy
- UV/VIS absorption spectroscopy and FTIR spectroscopy measurement of chemistry
- Schlieren imaging and Moire deflectometry for temperature and density measurements
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