Robust Hydrogen Peroxide Enhanced Plasma Effluent for the Clinical Setting

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Outline

- Non-thermal Plasma in the Clinical Setting
- $\text{H}_2\text{O}_2$ Enhanced Plasma Effluent
- Sterilization/Disinfection Results
- Frequency Comb Spectroscopy of Effluent
Non-thermal Plasma in the Clinic

CLINICAL NEEDS

High Capital Cost/Low Throughput
- Cancer
- Dermatology, skin diseases
- Cell apoptosis

Low Capital Cost/High Throughput
- Sterilization of instruments/personal articles
- Wound infections
- Diabetic wounds
- Surgical site preparation

NON-THERMAL PLASMA TECHNOLOGIES

Specialized Configurations
- “Direct” Treatments [Fridman et al., 2008]
- Argon/Helium Plasmas [Ermolaeva et al., 2011; Laroussi et al. 2006]

Robust Configurations
- “Indirect” Treatments [Burts et al., 2009]
- H$_2$O$_2$ Enhanced Plasma Effluents [Watts et al, 2006]
Indirect Plasma Effluent Development

Microwave Discharge → Microwave Discharge + Water → Microwave Discharge + H₂O₂ → Dielectric Barrier Discharge + H₂O₂
Device Block Diagram

- **STERILIZATION CHAMBER**
  - (30 C, 1 ATM)
  - Coaxial, 9 kV; 24 kHz, 2 cm OD, 1mm gap

- **MAIN PUMP**
  - ~10 ℓ/min

- **DBD DISCHARGE**
  - Up to 3 m
  - H₂O₂

- **HEPA FILTER ACTIVATED CARBON**

- **EXHAUST PUMP**
Different components

Bacillus Atrophaes Spore Discs (10^6 CFU, n = 6)

Percent Kill Rate

Control | Plasma Only | Plasma + Water | H_2O_2 Only | Plasma + H_2O_2
Sterilization of Agar Plates

6 log reduction in 15 seconds for *Staphylococcus aureus*!

6 log reduction in 60 seconds for *Pseudomonas*
Sterilization of ID Badges

Plasma treatment reduces bacterial contamination of badges. Badges containing $10^6$ CFU of Staphylococcus aureus (SA) or Pseudomonas (PsA) were exposed to plasma treatment for the indicated times. Dampened cotton swabs were used to remove the bacteria and placed in 2ml LB medium following plasma treatment and the cultures were serially diluted in PBS, spotted on LB agar plates and the viable bacteria enumerated after overnight incubation at 37°C. Data are presented as % bacterial cell recovery +/- SD. *** ($P < 0.001$) compared to SA or PsA controls by two-way ANOVA of transformed data with Bonferroni's post-test.

6 log reduction in 15-60 seconds for *Pseudomonas*

6 log reduction in 60 seconds for *Staphylococcus aureus*

15 seconds enough for 99% kill rate
**Escherichia coli** Biofilms

5 minute exposure

- **Well 1**: ~100% (6.59) covered
- **Well 2**: 99.9% (3.05)
- **Well 3**: 99.5% (2.28)
- **Well 4**: 99.7% (2.49)
- **Well 5**: 0.5% (0.0002)
- **Well 6**: 45.9% (0.27)

![Graph showing E. coli Biofilms 5 Min Exposure](image)

The graph illustrates the recovered CFUs for each well after a 5-minute exposure. The x-axis represents well number (0 to 6), and the y-axis represents the recovered CFUs, with a range from $10^0$ to $10^{12}$. The bars for each well show the recovered CFUs, with Well 1 having the highest recovery (approximately 100%) and Well 5 having the lowest recovery (0.5%).
Direct Frequency Comb Spectroscopy

Source of light: Optical Frequency Comb
- broadband, high-resolution, high power (max resolution 150MHz, 1W output power)
- simultaneous bandwidth 0.3 um – 45 000 lasers in one beam !!
- tunable between 2.8 um and 4.8 um, range of strong molecular absorption

High-finesse optical cavity / Multipass cell
- high sensitivity to absorption

Broadband spectral readout
- rapid data acquisition (time resolution 2 s)
- Fourier Transform spectrometer

Direct Frequency Comb Spectroscopy
Plasma Effluent Measurements

Synchronously pumped OPO as tunable source of light

Two bands

\[ 2700-2900 \text{ cm}^{-1} \ [\text{O}_3, \text{N}_2\text{O}, \text{NO}_2] \]

\[ 2620-2820 \text{ cm}^{-1} \ [\text{H}_2\text{O}_2, \text{O}_3, \text{N}_2\text{O}] \]
Concentrations Without H$_2$O$_2$ Solution

![Graph showing concentrations of O$_3$, N$_2$O, and NO$_2$ over time with pump on and plasma on and off events.]
Concentrations with H$_2$O$_2$ Solution

- **O$_3$**
- **H$_2$O$_2$**
- **N$_2$O**
- **NO$_2$**

Time (s): 0 - 50
Concentrations Summary

### Without H\textsubscript{2}O\textsubscript{2} Solution

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<tr>
<td>O\textsubscript{3}</td>
<td>756 ± 6 ppm</td>
<td>H\textsubscript{2}O\textsubscript{2}</td>
<td>6 ± 2 ppm</td>
<td>N\textsubscript{2}O</td>
<td>24.0 ± 0.6 ppm</td>
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### With H\textsubscript{2}O\textsubscript{2} Solution

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<tr>
<td>O\textsubscript{3}</td>
<td>394 ± 14 ppm</td>
<td>H\textsubscript{2}O\textsubscript{2}</td>
<td>415 ± 14 ppm</td>
<td>N\textsubscript{2}O</td>
<td>16 ± 3 ppm</td>
</tr>
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**Plots:**

- **O\textsubscript{3}, H\textsubscript{2}O\textsubscript{2} concentrations [ppm]**
- **N\textsubscript{2}O, NO\textsubscript{2} concentrations [ppm]**

**Legend:**

- **O\textsubscript{3}**
- **H\textsubscript{2}O\textsubscript{2}**
- **N\textsubscript{2}O**
- **NO\textsubscript{2}**

**Time (s):**

- **Plasma on**
- **Plasma off**

**Concentrations Summary:**

- **Without H\textsubscript{2}O\textsubscript{2} Solution**
  - O\textsubscript{3} = 756 ± 6 ppm
  - H\textsubscript{2}O\textsubscript{2} = 6 ± 2 ppm
  - N\textsubscript{2}O = 24.0 ± 0.6 ppm
  - NO\textsubscript{2} = 18.9 ± 0.1 ppm

- **With H\textsubscript{2}O\textsubscript{2} Solution**
  - O\textsubscript{3} = 394 ± 14 ppm
  - H\textsubscript{2}O\textsubscript{2} = 415 ± 14 ppm
  - N\textsubscript{2}O = 16 ± 3 ppm
  - NO\textsubscript{2} = 15 ± 1 ppm
Other Species

- **OH**: could not be measured because of overlap of absorption lines with water
- **O**: Lack of strong absorption lines in mid IR band
- **NO**: absorption line at 2.8 μm overlaps with water, line at 5 μm out of range
Applications and Future Work

• Optimization of Effluent/ Treatment of Infected Wounds
  – Diabetic wounds, burn wounds

• Show that no adverse effects to skin
  – Mouse experiment protocol approved (already verified with earlier microwave discharge/H\textsubscript{2}O\textsubscript{2} system)

• Use electron spin resonance (ESR) to determine penetration depth of radicals into tissue
New Plasma Textbook

- Targeted at engineering students
- Intuitive explanations
- Mathematical thoroughness and completeness
- Organizational charts
- Solution manual and lecture slides
References