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FAST TRACK COMMUNICATION

Long-term antibacterial efficacy of air plasma-activated water

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Abstract
Indirect air dielectric barrier discharge in close proximity to water creates an acidified, nitrogen-oxide containing solution known as plasma-activated water (PAW), which remains antibacterial for several days. Suspensions of *E. coli* were exposed to PAW for either 15 min or 3 h over a 7-day period after PAW generation. Both exposure times yielded initial antibacterial activity corresponding to a ∼5-log reduction in cell viability, which decreased at differing rates over 7 days to negligible activity and a 2.4-log reduction for 15 min and 3 h exposures, respectively. The solution remained at pH ∼2.7 for this period and initially included hydrogen peroxide, nitrate and nitrite anions. The solution composition varied significantly over this time, with hydrogen peroxide and nitrite diminishing within a few days, during which the antibacterial efficacy of 15 min exposures decreased significantly, while that of 3 h exposures produced a 5-log reduction or more. These results highlight the complexity of PAW solutions where multiple chemical components exert varying biological effects on differing time scales.

(Some figures may appear in colour only in the online journal)

1. Introduction

It is known that air plasmas created by gliding arc or dielectric barrier discharge (DBD) adjacent to non-buffered liquid water near room temperature and at atmospheric pressure will generally create acidic (∼pH 2–3) solutions that contain hydrogen peroxide (H_2O_2), nitrate (NO_3^-) and nitrite (NO_2^-) anions, as well as other species [1]. Further, it has been reported by several groups that this acidic solution is effective in killing bacteria in suspension [2–5]. If the pH rises above about 3–4, the antibacterial effectiveness is known to drop significantly [1].

Aqueous solutions of nitrite ions at pH below 4–5 are known to be antimicrobial. So-called 'acidified nitrite' has received a great deal of attention in the biomedical literature due to the generation of nitric oxide (NO) from the decomposition of nitrous acid. Nitrite ions are thought to play a significant biological role as an intermediate in the path from dietary nitrate to nitric oxide. Lundberg *et al* [6] review the multiple ways the coupled nitrate, nitrite and nitric oxide pathways are involved in physiology, underlying promising therapies for conditions including heart attack, stroke, hypertension and gastric ulceration. Among a long list of applications, including acidified nitrite creams for use as topical NO-donating wound healing agents [7], concentrated acidified nitrite solutions were shown to be very effective in surface disinfection, even for *C. difficile* spores [8]. Kono *et al* [9] note the synergistic antimicrobial effects of nitrite, H_2O_2 and low pH.

We hypothesized that long-lived secondary products, such as H_2O_2, nitrite and nitrate, would likely be responsible for the extended biological effects of plasma-activated water (PAW) after plasma treatment. In this paper, we report measurements of the correlation between the manner in which PAW is created via indirect air DBD, the solution composition, storage conditions, duration of bacterial exposure and antibacterial effectiveness for a period of up to 7 days after creation.

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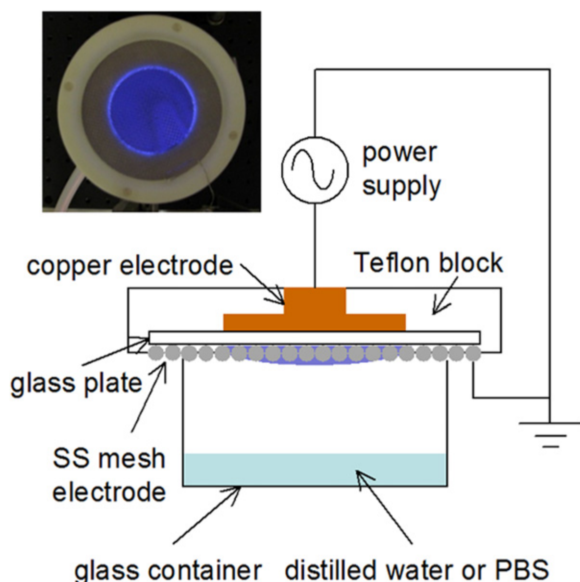

Figure 1. Experimental setup for PAW/PAPBS generation by the indirect air DBD. The inset shows visible image of the discharge.

2. Experimental

Figure 1 shows our experimental system for PAW generation. We employed surface micro-discharge similar to a previous report [10]. A powered electrode is made of a copper plate of 47 mm diameter and the ground electrode is stainless steel woven wire mesh (0.5 mm wire diameter and 8 × 8 meshes per cm). A quartz plate of 1 mm thickness was used between the powered and ground electrode. A function generator (Protek, Model 9301) and high voltage amplifier (Trek, 10/40A) were used to generate high voltage of 5 kV in magnitude and a high frequency of 10 kHz continuous sinusoidal wave. The power consumption was 5 W (0.288 W cm^{-2}). Ten ml of distilled water (Invitrogen, UltraPure Distilled Water) and phosphate buffered saline (PBS; 10mM sodium phosphate, 0.9% NaCl at pH 7.4) were added to a glass container of 70 mm diameter, covered by the electrodes and treated for 20 min to create PAW. The gap between the ground electrode and the solution surface was 42 mm.

E. coli K12 were cultured in Lysogeny Broth (LB) growth medium to an optical density at 600 nm of 1.0, corresponding to a bacterial concentration of approximately 10^8 cfu ml^{-1}. The culture was incubated for 18 h at 37 °C and shaken at 200 rpm. Aliquots of the culture (100 μl) were pelleted by centrifugation at 5000 rpm for 10 min. The pellet was resuspended in either 1 ml of distilled water, PBS, PAW or plasma-activated PBS (PAPBS) to determine each solution's antimicrobial effect [5]. The suspensions were incubated for either 15 min or 3 h. In the latter case, suspensions were vortexed every 20 min to ensure thorough mixing. At the end of the 15 min or 3 h incubation, the suspensions were immediately diluted in PBS. Tenfold serial dilutions of each suspension were plated on LB agar and incubated overnight, after which colonies were counted to determine the number of viable cells. The antibacterial effect of treated solutions was quantified by the log reduction, $\log(N_0/N)$, where N_0 is the number of viable cells after exposure to either untreated distilled water or PBS and N is the number of viable cells after exposure to PAW or PAPBS. The maximum log reduction detectable using this approach is approximately 5–6. In some cases discussed below, the actual log reductions could have been higher than the reported values.

H_2O_2 was measured with a biochemistry analyser (YSI, 2700 Select). Nitrite and nitrate were quantified via UV absorbance spectroscopy (Molecular Devices, Spectramax M2). Untreated distilled water and PBS were used as blanks for measurements in PAW and PBS, respectively. Concentration standards for nitrite and nitrate were generated with sodium nitrite and sodium nitrate, and prepared at the measured pH of PAW and PAPBS. A linear regression was applied to the absorbance scans of nitrite and nitrate from 200 to 400 nm to obtain extinction coefficients for each wavelength.

3. Results and discussion

The antimicrobial actions of PAW were examined over periods of several days along with the composition and antimicrobial activity over the same time scale. The solution pH, composition, and antimicrobial effects were also measured just after plasma exposure, and these were found to be similar to results reported by Oehmigen *et al* [1]. Oehmigen *et al* [1] used normal saline and PBS-buffered saline solutions whereas we began with distilled water. No significant changes in antibacterial efficacy were observed in comparisons of PAW generated from distilled water or saline solutions. Additionally, no death was observed when cells were incubated with untreated distilled water (data not shown).

Figure 2(*a*) shows the antibacterial efficacy when cells were exposed to PAW for 3 h and figure 2(*b*) corresponds to a 15 min exposure. Figure 2(*c*) represents antibacterial efficacy of PAPBS for 3 h incubation. For all solutions, the pH was constant over the experiment at 2.7±0.2 for PAW and 7.0±0.1 for PAPBS. The pH of 2.7 is close to the reported pK_a 2.8–3.2 for nitrous acid [11]. Little bacterial death was observed when *E. coli* was treated with all PAPBS solutions, while a ∼5-log or greater reduction in bacterial viability was measured in both 15 min and 3 h exposures with PAW directly after plasma treatment. The antibacterial efficacy of 3 h exposures with PAW remained at or above 5 logs for 2 days then decreased to 2.4 logs after 7 days, while the efficacy of 15 min exposures dropped from 5.6 logs to 2.4 logs in 30 min, remained at about 1 log at the end of day 1 and day 2, then yielded no antibacterial activity after 7 days. The antibacterial assay detection limit is within the error bars of the measured value for the first 2 days of the 3 h exposures; thus, the exact rate at which the antimicrobial efficacy declines over this time is unclear. As noted above, it is possible that the initial reduction following 3 h incubation is greater than 5 logs.

Kamgang-Youbi *et al* [4] investigated the antibacterial activity of water after treatment with a gliding arc. These researchers found a substantial reduction in viable *H. alvei* cells upon exposure to PAW, and this activity persisted for at least 24 h. Additionally, Oehmigen *et al* [1] found that plasma treated saline solutions aged for 30 min were still

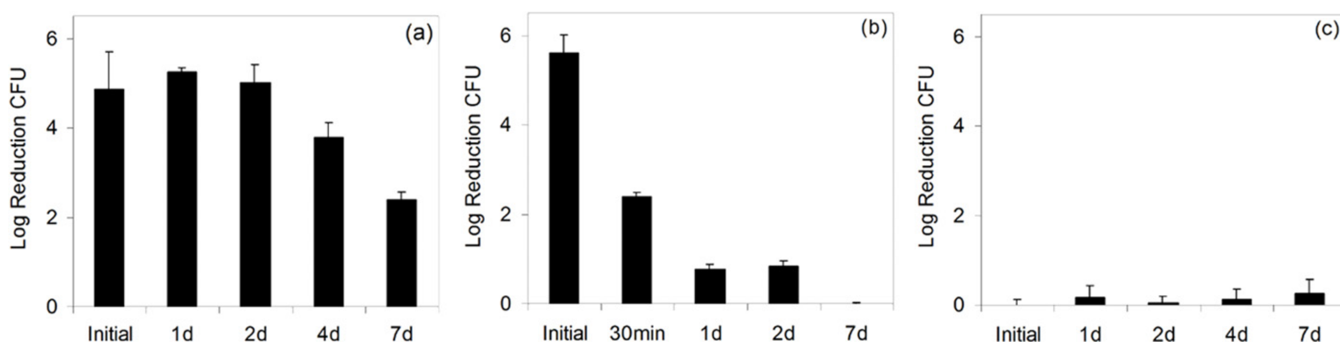

Figure 2. Time-dependence of antibacterial activity of PAW incubated with *E. coli* for (*a*) 3 h, (*b*) 15 min, and (*c*) of PAPBS incubated for 3 h after indirect air DBD treatment.

bactericidal when cells were exposed for 15 min. However, these authors found that the antibacterial efficacy was greatly reduced after 30 min. We observed similar behaviour in which 15 min exposures yielded a drop in efficacy from 5.6 to 2.4 logs following a 30 min delay between creation of PAW and incubation with bacteria.

Figure 3 shows UV absorbance data and concentrations of nitrites, nitrates and H_2O_2. H_2O_2 is known to be involved in the antimicrobial properties of plasma treated solutions [3, 12]. Previous investigations seeking to define the antimicrobial contributions of species in PAW have attributed different potencies to H_2O_2. Burlica *et al* [3] found that approximately 3mM H_2O_2 contributed a 2-log reduction in bacterial colony formation, while Naitali *et al* [12] found that 10μM acidified H_2O_2 yielded 0.4-log reduction. We measured an intermediate concentration of approximately 100μM H_2O_2 in PAW (figure 3(*e*)), which decreased to below the detection limit ($\sim$1–5μM) over 2 days. PAPBS solutions exhibited a concentration of approximately 200μM H_2O_2 that did not vary significantly over 7 days.

UV absorbance data were acquired for each plasma treated solution to quantify nitrite and nitrate concentrations as described above. The absorbance scans of PAPBS, and thus the concentrations of nitrite and nitrate, were effectively constant over 7 days; however, the UV absorbance of PAW varied greatly (figure 3(*a*)). Nitrite levels in PAW decreased from 1.2 mM to 2 μM within two days, while the nitrate concentration increased quickly from 1.2 to 3 mM then increased more slowly over the following 4 days (figure 3(*f*)).

As noted above, the antibacterial activity of acidified nitrite is well known and is presumably due to the decomposition of nitrous acid to generate nitric oxide [6]. Several groups have studied the contribution of acidified nitrite to the antimicrobial capacity of PAW. Naitali *et al* [12] attributed a $\sim$3-log reduction to acidified nitrite and a 5-log reduction to the synergistic effect of acidified nitrite with H_2O_2 and nitrate. This synergy presumably arises from the formation of reactive species such as peroxynitrous acid, peroxynitrite, and related nitrogen-oxide products. This increase in antibacterial efficacy of nitrite and H_2O_2 at acidic pH presumably accounts for the enhanced antimicrobial action of acidic PAW over neutral PAPBS. We measured an initial concentration of 1.2 mM nitrite in PAW that decreased quickly over 2 days to 2 μM, during which the antibacterial efficacy of PAW was at or above 5 logs in 3 h exposures, but dropped significantly in 15 min exposures. It should be noted that nitrogen-oxide solution chemistry is far from fully determined. The complexity of this chemistry is evident from a relatively recent review on peroxynitrite by Goldstein *et al* [13].

A peak was observed at 262 nm in the PAW samples but not PAPBS (figure 3(*a*)). A Gaussian function fit well to this peak and the calculated peak height increased linearly over the experiment. The peak at 262 nm was only present when PAW samples were stored in sealed tubes and did not appear in samples stored in open tubes, suggesting the involvement of a gas phase species diffusing either into or out of the PAW solution. No difference was observed, however, in the antibacterial capabilities of PAW samples stored in open or closed tubes (data not shown). Many organic nitro molecules exhibit UV absorbance maxima around 260 nm [14]. Thus, the nitration of an organic contaminant could account for this peak. A similar peak was observed previously in PAW [15].

4. Concluding remarks

In summary, the antimicrobial actions of PAW were monitored over periods of several days. The H_2O_2 and nitrite levels correlated with bacterial log reductions from 15 min exposures with cells, but not from 3 h exposures, which yielded higher and sustained antibacterial efficacy that persisted for at least 7 days. These results highlight the complexity of PAW solutions where multiple chemical components exert varying biological effects on differing time scales. Further study will more completely elucidate the nature and time-dependent concentration profile of other species in PAW.

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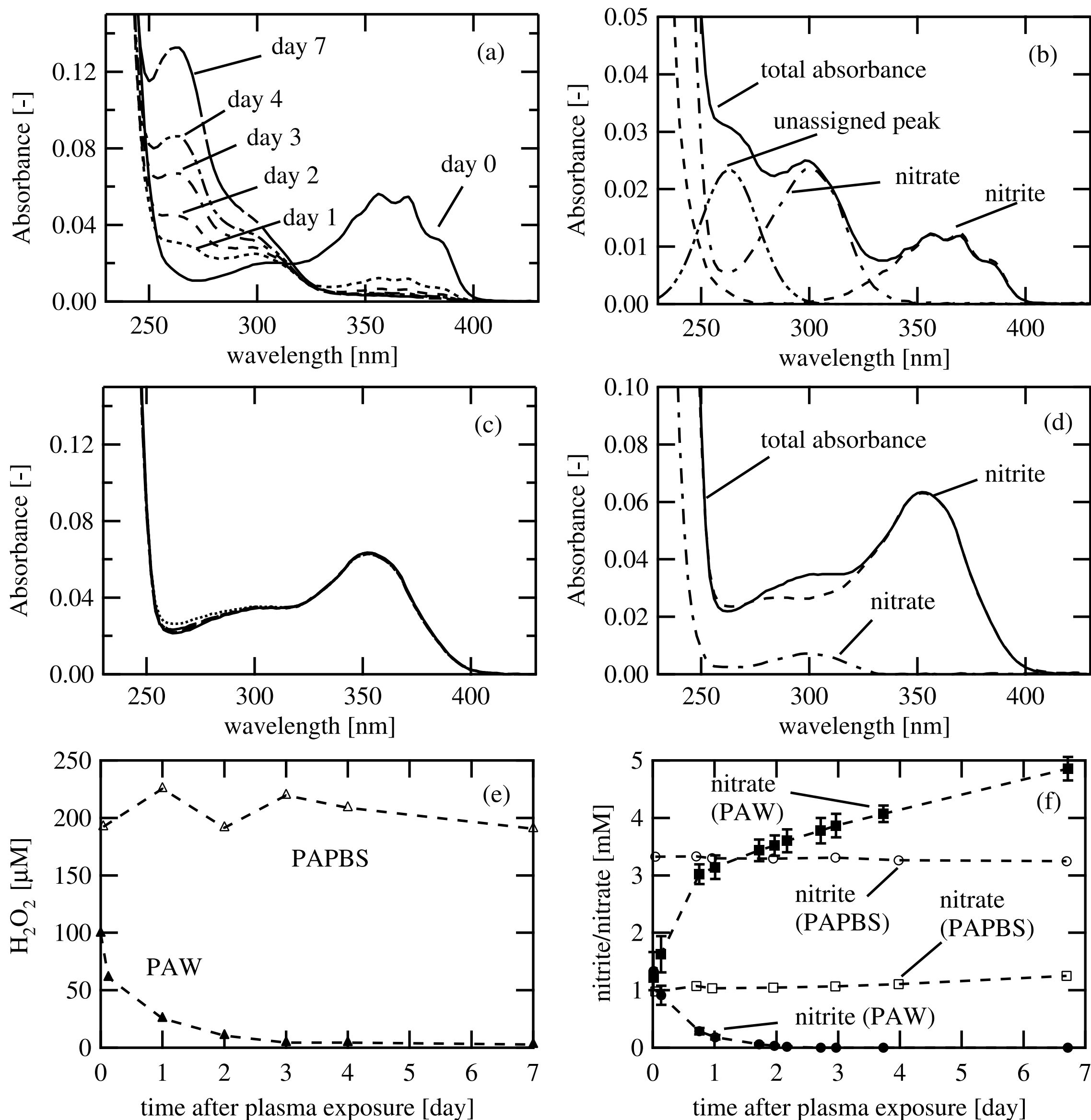

Figure 3. UV absorbance of (*a*) PAW and (*c*) PABS over 7 days after indirect air DBD treatment. Note that all the lines overlap for PAPBS (*c*).The representative spectra one day after air DBD treatment for (*b*) PAW and (*d*) PAPBS. The concentration of (*e*) H_2O_2 and (*f*) nitrites/nitrate in PAW and PAPBS.

All opinions expressed in this paper are the author's and do not necessarily reflect the policies and views of DHS, DOE or ORAU/ORISE.

References

[1] Oehmigen K, Hähnel M, Brandenburg R, Wilke C, Weltmann K and von Woedtke T 2010 *Plasma Process. Polym.* **7** 250
[2] Burlica R, Kirkpatrick M and Locke B 2006 *J. Electrostat.* **64** 35
[3] Burlica R, Grim R, Shih K and Balkwill D and Locke B 2010 *Plasma Process. Polym.* **7** 640
[4] Kamgang-Youbi G, Herry J-M, Bellon-Fontaine M-N, Brisset J-L, Doubla A and Naïtali M 2007 *Appl. Environ. Microbiol.* **73** 4791
[5] Kamgang-Youbi G, Herry J-M, Brisset J-L, Bellon-Fontaine M-N, Doubla A and Naïtali M 2008 *Appl. Microbiol. Biotechnol.* **81** 449
[6] Lundberg J, Weitzberg E and Gladwin M 2008 *Nature Rev. Drug Discovery* **7** 156
[7] Weller R and Finnen M 2006 *Nitric Oxide* **15** 395
[8] Wullt M, Odenholt I and Walder M 2003 *Infect. Control Hosp. Epidemiol.* **24** 765
[9] Kono Y, Shibata H, Adachi K and Tanaka K 1994 *Arch. Biochem. Biophys.* **311** 153
[10] Morfill G E, Shimizu T, Steffes B and Schmidt H-U 2009 *New J. Phys.* **11** 115019
[11] Riordan E, Minogue N, Healy D, O'Driscoll P and Sodeau J R 2005 *J. Phys. Chem.* A **109** 779
[12] Naitali M, Kamgang-Youbi G, Herry J-M, Bellon-Fontaine M-N and Brisset J-L 2010 *Appl. Environ. Microbiol.* **76** 7662
[13] Goldstein S, Lind J and Merenyi G 2005 *Chem. Rev.* **105** 2457
[14] Schroeder W A, Wilcox P E, Trueblood K N and Dekker A O 1951 *Anal. Chem.* **23** 1740
[15] Moussa D, Doubla A, Kamgang-Youbi G and Brisset J-L 2007 *IEEE Trans. Plasma Sci.* **35** 444