Supporting Information

Cold atmospheric plasma activated water as a prospective disinfectant: the crucial role of peroxynitrite

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Species	EDL (m)	Species	EDL(m)
N^+	1.1 × 10 ⁻⁷	H_2O_2	>0.01
N_2^+	2.2×10^{-7}	HO_2	3.7×10^{-4}
N_3^+	7.4×10^{-7}	ОН	1.3×10^{-4}
N_4^+	7.0×10^{-8}	0	6 × 10 ⁻⁵
NO^+	3.0×10^{-5}	O_3	>0.01
NO_2^+	7.1 × 10 ⁻⁵	$O_2(a^1\Delta)$	4.6×10^{-4}
N_2O^+	4.5×10^{-7}	HNO ₃	>0.01
NO-	3.6×10^{-7}	HNO_2	>0.01
NO ₂ -	9.4 × 10 ⁻⁶	NO	2.0×10^{-4}
NO ₃ -	6.2 × 10 ⁻⁵		
N_2O^+	6.7 × 10 ⁻⁵		

Table S1. Estimated diffusion length of several species.^{S1,S2}

The efficacy of plasma activated water is determined by the depth of diffusion of gas-phase plasma generated-species into liquid. According to the Einstein-Smulochowski equation, i.e. $EDL = \sqrt{6D_g \tau}$, with D_g being the diffusion coefficient and τ the lifetime of a species, the effective diffusion distance in lifetime (EDL) of species could be estimated. The EDL of these involved species are listed in **Table S1**. The lifetime of H₂O₂ is ~10⁴ s in atmosphere and the effective diffusion distance EDL is > 0.01m.^{S1,S2} Moreover, these long-lived species, like (H₂O₂, NO₂⁻, NO₃⁻) in solution could be stably existed for several days or even years under a certain condition. Singlet delta oxygen (SDO), O₂($a^{1}\Delta_{g}$), is the first electronically excited state of molecular oxygen (O₂), having an excitation energy of 0.98 eV, which has been recognized as a species playing a major role in atmospheric pressure plasma chemistry, biology, medicine and plasma-assisted combustion. However, according to the Einstein-Smulochowski equation, the EDL of SDO in the air gap was estimated to be less than 0.02 cm, indicating a short

penetration depth of the dissolved singlet delta oxygen. Therefore, the contribution of gasphase singlet delta oxygen in PAW reactivity is not significant in our work.

An atmospheric-pressure plasma jet similar to that reported in literature^{S3,S4} was used to generate PAW in a continuous flow system, as shown in **Fig. S1**. Plasma was ignited between a tungsten steel tube (1.02 mm and 6.35 mm in inner and outside diameters, respectively) and an aqueous solution surface using a custom-made AC power source. A 10 k Ω resistor was connected in series with the tungsten steel electrode to avoid the transfer of plasma from glow-like discharge to arc. Air was added into the discharge tube from the top of the device at the flow rate of 20 sccm. A graphite rod (5 mm in diameter) was placed at the bottom of solution to act as an inert cathode electrode (positive voltage applied to the tungsten steel electrode) and the discharge gap was 3 mm. Plasma interacts with media through the gas-liquid interface, thus to ensure uniform activation of liquid media, a total of 50 mL of liquid was circulated by a peristaltic pump at a flow rate of 200 ml/min.

The voltage between the tungsten steel and graphite electrodes was measured by a high voltage (H.V.) probe (Tektronix P6015A) and the current was estimated by dividing the voltage across a 10 Ω resistor, which was connected in series with the graphite electrode. Optical emission spectra (OES) were obtained using a SpectraPro-750i monochromator (Acton Research Corporation) with a resolution of 0.5 nm in the wavelength range of 300-800 nm to identify major excited reactive species in gas phase during PAW generation by the air plasma jet. The optical fiber was located near the liquid surface and 20 mm away from the plasma. A molecular-beam mass spectrometer (MBMS, Hiden EQP mass/energy analyzer HPR 60) was operated in the time-averaged mode to provide clues for chemical and physical processed in the gas-liquid interphase. The distance between the bottom of plasma and orifice of the mass spectrometer was 5 mm. Based on the discharge current and voltage curves, the average power

consumption of PAW generation was estimated to be ~ 0.3 kWh per litre with the needed bactericidal activity.



Fig. S1 (a) The schematic diagram of the continuous flow system used in this study, and (b) a photograph of the plasma-liquid interactions producing PAW. Reproduced with permission [S3]. Copyright 2018, Elsevier.



Fig. S2 Schematic of the experimental procedures for reactive species quantification.

Folic acid was implied as a fluorescent probe for peroxynitrite determination in PAW. Folic acid (SIGMA-ALDRICH, Brisbane, Australia) solution was prepared by dissolving appropriate amount of folic acid in 1 mmol·L⁻¹ NaOH and kept frozen, and the working barbital buffer solution was prepared by dissolving 4.125 g barbital sodium in 500 mL distilled water and add 0.7 mL 1.0 mol·L⁻¹ HCl. Peroxynitrite standard solutions were synthesized and the absorbance was detected by UV spectrometry at 302 nm ($\epsilon = 1670 \text{ mol}^{-1}\text{ cm}^{-1}$) according to the description explained in detail in the following figure referred to the method reported by Huang et al.⁸⁵

NaNO₂ $\xrightarrow{\text{mixed with H}_2O_2/\text{HCl}}$ Mixture $\xrightarrow{\text{filtered and}}$ Peroxynitrite (Sodium nitrite) $\xrightarrow{\text{quenched with NaOH, adding MnO}_2 \text{ powder}}$ Mixture $\xrightarrow{\text{filtered and}}$ Peroxynitrite Scheme 1. Synthesis of peroxynitrite.

The fluorescence spectra and relative fluorescence intensity were measured with a fluorescence-4500 (Hitachi, Japan) with a 10 mm quartz cuvette, the excitation and the emission wavelength slits were respectively set at 5.0 and 10.0 nm. Briefly, in a set of 10 mL-volumetric tubes containing pH 9.4 barbital buffer solution, 1.0 mL of folic acid (0.1 mmol·L⁻¹) and PAW were added. The tubes were closed and then quickly and carefully shaken. The reaction solution was kept at room temperature for 5 min. The fluorescence intensity of the solution was recorded at 460 nm with the excitation wavelength set at 380 nm. The fluorescence increment showed a linear relationship with the concentration of peroxynitrite in the range of 0 to 3.2 µmol/L with a correlation coefficient (R^2) of 0.9999 (**Fig. S3**).



Fig. S3 (a) Change in fluorescence intensity of the folic acid solution with increasing equivalents concentration of peroxynitrite, and (b) fluorescence intensity at 460 nm vs concentration of peroxynitrite. $\lambda_{ex} = 380$ nm, $\lambda_{em} = 460$ nm.



Fig. S4. (a) Proposed chemical reaction relation between long- and short-lived species tested in this work. (b) Biochemistry of peroxynitrite and mechanisms of cell death induced by peroxynitrite. Reproduced with permission [S6]. Copyright 2007, Springer Nature.



Fig. S5. Relationship of the concentration of peroxynitrite and bacteria inactivation in a function of different concentration of nitrite.

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