Water activated by non-thermal air plasma discharges for bio-decontamination and bio-medical effects

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1. Introduction and motivation

Biomedical effects of cold atmospheric pressure plasmas are typically mediated through water and aqueous solutions that are natural to biological cells. Cold plasmas generated in gases in contact with liquids produce reactive species that penetrate through the gas-liquid interface and initiate reactions leading to the secondary reactive species formation in the liquid. Air plasma – water interactions lead to the formation of reactive oxygen and nitrogen species (RONS) such as ozone O_3, hydroxyl (OH) radicals, and nitrogen oxides, (mainly NO and NO_2). Once they get into water solutions they generate hydrogen peroxide H_2O_2, nitrites NO_2^-, nitrates NO_3^-, peroxynitrites/peroxynitrous acid $ONOO^-$ /ONOOH. This is typically accompanied by chemical changes (acidification) and antimicrobial effects [1-3]. Peroxynitrites are powerful oxidants that can oxidize many cellular components and together with the acidic pH are hypothesized to be responsible for the strong bactericidal effect that can be maintained in the plasma activated water (PAW) for several hours/days after plasma treatment. [2-5].

2. Water activation by air plasma discharges

A simple way how to efficiently activate water by cold plasma discharges is to generate the plasma discharge between the high voltage (stressed) electrode and the water surface as the other discharge electrode (typically grounded). In our flowing water system, the grounded electrode is inclined and the treated water circulates on its surface (Fig. 1, left). Even more efficient water activation can be achieved by a combination of water electrospray with the discharge. Our water spray system enables the water flow directly through the high-voltage needle electrode into the

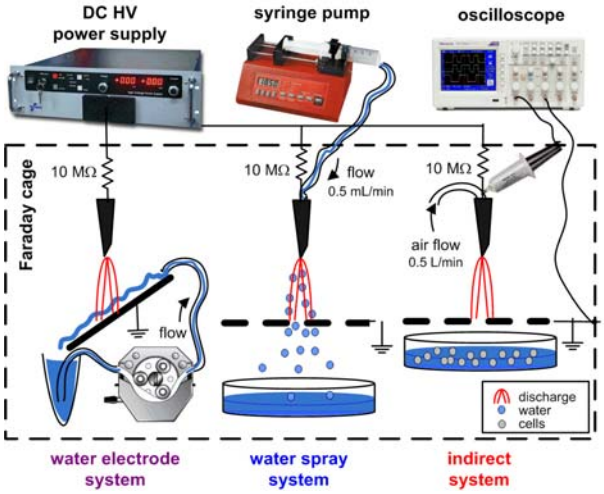

Fig. 1 Water electrode / water electrospray and indirect treatment of water by air transient spark discharge.

active discharge region, where it forms a spray of micrometric droplets (Fig. 1, center). The interaction of plasma with water droplets allows for very efficient mass transfer of plasma-generated reactive species into water [5-6]. Direct exposure of treated water to the discharge was compared with the indirect exposure by the discharge activated gas flow (Fig. 1, right). Self-pulsing DC-driven positive transient spark (TS) discharge operated in air was used as the non-thermal plasma source [7]. The electrical properties of this discharge can be easily controlled by the applied voltage and circuit parameters, which then control the production of active species, such as NO and NO_2 in the gas [8].

3. Diagnostics of plasma activated water

Detection of reactive species generated in the plasma activated water (PAW) is challenging due to the presence of highly reactive, short-lived species and possible cross-reactivities. It is therefore crucial to either develop new methods of their detection or adapt the existing methods

used in analytical chemistry and biology for specific conditions in PAW.

We focused on the detection of RONS formation induced by air plasma gas-liquid chemistry in PAW. Hydrogen peroxide, nitrites, nitrites, peroxynitrites and dissolved ozone were detected in PAW solutions (produced in water electrode or water spray systems) by colorimetric absorption and fluorescence spectroscopy and verified by ion chromatography or HPLC where specific oxidative and nitrosative products of phenol degradation were detected. Water solutions with different initial pH and conductivities were differentiated according their buffering capacity, pH or content of chlorine. The temporal evolutions of chemical reactions in water were also investigated in synthetically prepared aqueous solutions mimicking PAW.

4. Bactericidal and bio-medical effects of PAW

4.1 Bacteria in water

Bactericidal effects induced in water activated by the TS discharge in air (both in water electrode or water spray systems) were investigated. Inactivation efficiency of *E. coli* bacteria in water was determined in dependence on pH (controlled by buffers) and correlated with chemical changes induced in water and generation of RONS. The degree of inactivation and oxidative damage of bacteria increased with the decreasing pH. NO_2^- interact with H_2O_2 in acidic conditions and lead to the peroxynitrites (ONOO-/ONOOH, detected by fluorescence spectroscopy) that were identified as the most important bactericidal RONS agents in PAW [2,5]. Since PAW loses its bactericidal effects within a few hours, we also tested the possibility of its preservation by freezing [9].

4.2. Bacterial biofilms on surfaces

In low power air discharges with water electrospray, the bactericidal effect of ozone dissolved in water may play an important role. The effect of micrometric droplets formation in the water electrospray of very low flow rates contributed to a significant enhancement of the *E. coli* biofilm reduction on glass surfaces when treated by positive streamer and negative Trichel pulse corona discharges. Confocal laser scanning microscopy with various fluorescent staining showed enhanced biofilm etching when water was sprayed onto its surface [10]. Biofilm resistivity to common antibiotics and antibacterial chemicals is a serious issue responsible for most of nosocomial (hospital-acquired) infections. A cold plasma treatment (combined with water electrospray) represents a very promising method to biofilm eradication.

2.3 Eukaryotic cells

Both direct exposure TS systems, as well as indirect treatment resulted in cytotoxic effects on eukaryotic cancerous (HeLa) and normal (Vero) cells (*in-vitro*). The analysis of the cell viability, the induction of apoptosis and modification of the cell cycle shows that air plasma can selectively target cancerous cells, which is very important for possible future development of new plasma therapeutic strategies in cancer medicine [11].

Acknowledgments

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