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DC TRANSIENT SPARK DISCHARGE IN WATER: EFFECTS ON CELLS, DNA, PROTEINS AND ENZYMES

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DC-driven transient spark (*TS*) discharge operated in air in contact with water (phosphate buffered solution (*PBS*) or deionized water) was used for the treatment of mammalian cells (*Vero line* normal cells, *HeLa line* cancerous cells), dsDNA, protein (*Bovine Serum Albumin*) and enzyme (*pepsin*). Two systems using different methods of water treatment were used: water electrospray and water electrode. Direct exposure of cells to the discharge and indirect exposure to discharge activated gas flow were compared, as well as the effects of *TS* discharge and pulsed plasma jet in helium used in parallel experiment. The cells were analyzed for viability and apoptosis. Viability of cells was evaluated by trypan/methylene blue staining, apoptosis by complementary analysis using Annexin V and Propidium iodide, while DNA, protein, and enzyme concentrations were evaluated by UV absorption and fluorescence spectroscopy.

1. Introduction

Cold atmospheric pressure plasmas generated by various electrical discharges have been successfully tested for various biological and biomedical applications over the past decade. Thanks to ionizations, dissociations, excitations, production of various chemically active species and reactions occurring at relatively low gas temperatures, the plasmas can efficiently kill bacteria, or even spores and biofilms that are generally very difficult to inactivate. Plasmas are very suitable for bio-decontamination, disinfection and sterilization of surfaces, medical instruments, water, air, food, and even living tissues. Despite many reported positive effects of the plasmas, their interaction with living cells and microorganisms remains still relatively not well understood.

2. Experimental

The objective of this work was to study the plasma interaction with living cells and selected biomolecules (DNA, protein, enzyme). The plasma was generated by DC-driven transient spark (*TS*) discharge in atmospheric pressure air [1] in contact with water – either phosphate buffered solution (*PBS*) or deionized water (*DI*). Elementary processes in the plasma were investigated and linked to the biophysical response of normal cells (*Vero line*, monkey kidney cells) and cancerous cells (*HeLa line*, human cervical cells) and effects on DNA, protein (*Bovine Serum Albumin*) and enzyme (*pepsin*). Direct plasma exposure was compared with an indirect exposure of the water to the plasma activated gas flow. The effects of *TS* discharge were compared with the results obtained with He pulsed plasma jet in parallel experiment.

Two systems of *TS* discharge using different methods of water treatment were used. The first system, *water electrode system (WE)*, with a point-to-plane discharge geometry consisted of high voltage needle electrode placed above the inclined grounded plane electrode (**Fig.1a**). The water was run through a narrow channel in the plane electrode and circulated by a peristaltic pump with various flow rates (up to 30 mL/min). The second system, *water electrospray system (WS)*, also with point-to-plane geometry, consisted of high voltage hypodermic needle placed above the grounded mesh electrode with water delivered into the discharge zone via the hollow needle with a constant flow rate (0.5 mL/min), depicted in **Fig. 1b** and described in detail in [2]. Both systems were operated in atmospheric pressure air, with electrode distance 1 cm, frequency up to 4 kHz and the treatment time up to 20 min. Direct exposure of cells to the *TS* discharge systems was compared to indirect exposure to discharge activated gas flow using the setup depicted in **Fig. 1c**. The reference system of the *pulsed*

plasma jet was of a dielectric barrier discharge type generated in He with the constant gas flow rate, described in detail in [3].

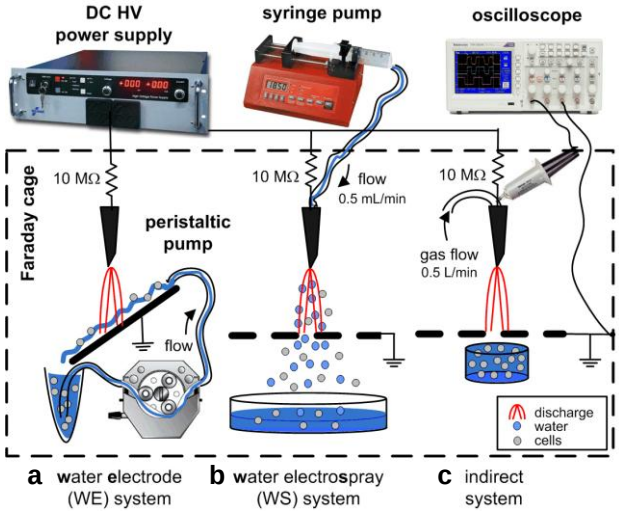

Fig. 1. Experimental setups of WE (a) and WS (b) systems for direct plasma exposure; and system for indirect exposure to the plasma activated gas flow (c).

The cells were analyzed for viability by trypan/methylene blue staining, and for apoptosis by complementary analysis using Annexin V and Propidium Iodide. The concentrations of DNA, protein, and enzyme were evaluated by UV absorption and fluorescence spectroscopy. The chemical analysis of treated water solutions was performed too and concentration of hydrogen peroxide, nitrites and nitrates, peroxynitrites, and dissolved ozone were measured, as well as acidity, conductivity and temperature of the solutions.

3. Results

This section presents the main results of direct and indirect plasma exposure of cells, DNA, protein and enzyme to *TS* discharge. **Fig. 2** shows the results of direct treatment of *TS* discharge on viability of HeLa cells. The initial concentration was approximately 500,000 cells/mL of *PBS*. Comparison of *WE* and *WS* systems (**Fig.2a**) shows similar lethal effect obtained in both systems. i.e. 51-56 % cytotoxicity. In general, cell viability decreased with increasing treatment time and discharge power. The maximal cytotoxicity of 93.5% was observed for 20 min treatment time and 4 kHz (**Fig.2b**).

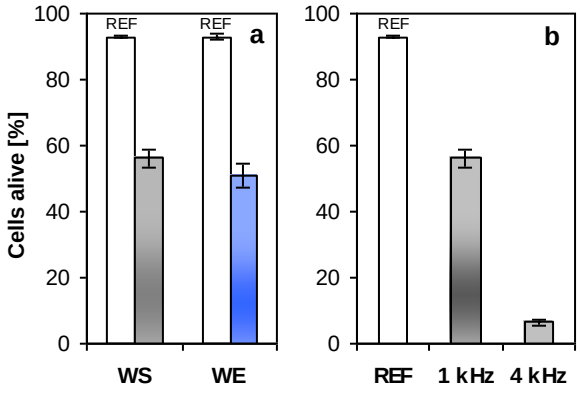

Fig. 2. Viability of HeLa line cells after direct exposure to *TS* discharge: **a**) comparison of *WS* and *WE* systems efficiency at 1 kHz after 10 min treatment, **b**) efficiency of *WS* system after 10 min at 1 Hz and 4 kHz.

Direct exposure of the cells to the discharge was compared with the indirect exposure of the cells to the discharge activated gas flow (30%O_2 in N_2, 0.5 L/min) delivered via syringe needle in point-to-plane geometry with mesh electrode placed 1-2 mm above the water surface. **Fig. 3** shows the viability of both Vero and HeLa cells after several minutes of indirect exposure. Stronger effect of plasma exposure was observed for HeLa (cancerous) cells than Vero (normal) cells. In general, lower cytotoxicity was observed for the indirect exposure compared with the direct exposure. In case of HeLa cells, 71% of cells were found still alive after 4 min of indirect exposure and 24 hours of incubation. The incubation is the time interval the cells were allowed to grow in an incubator after the plasma exposure to enter to the log phase, after which they were detached and re-suspended in *PBS*. Longer incubation time usually resulted in smaller overall cytotoxicity. Incubation time also affected the apoptotic behaviour of cells before their final death (**Fig. 4**).

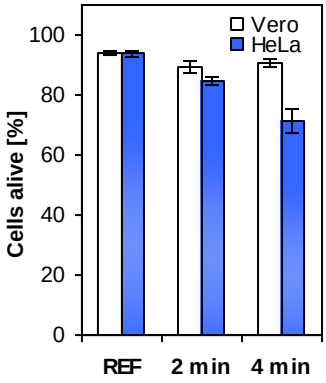

Fig. 3. Viability of Vero and HeLa line cells after 2 and 4 min of indirect *TS* plasma exposure [12.5 kV, 2 kHz].

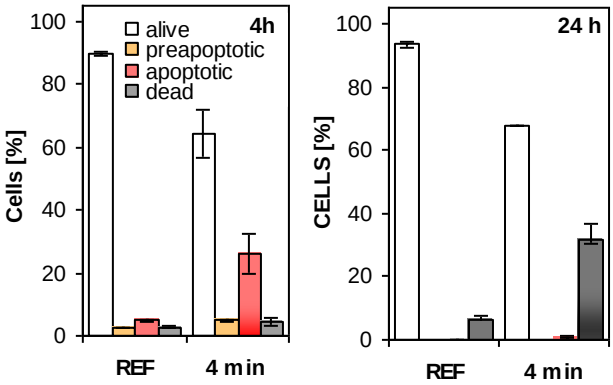

Fig. 4. Viability and apoptosis of HeLa line cells after 4 min of indirect treatment and 4h and 24 h of incubation [*TS*, 12.5 kV, 2 kHz].

The apoptosis was analysed by complementary tests using Annexin V and Propidium iodide. The results showed an increase of the number of apoptotic cells after 4 hours (26%), which were responsible for the increase of dead cells after 24 hours (32%). The effects of *TS* discharge and pulsed plasma jet were compared in parallel experiments. The jet was operated in helium (3 L/min) and used to treat HeLa and Vero cells for 4 min (applied voltage 4-8 kV, frequency 1-4 kHz). Maximum cytotoxicity of the plasma jet (29%, HeLa) was found comparable with indirect *TS* discharge exposure, but smaller compared to the direct exposure of *TS* discharge.

TS discharge was also used for the treatment of various biomolecules normally present inside the cells, including DNA, protein and enzyme. **Fig. 5a** shows the effect of *TS* discharge on the solution of DNA (9 ng/μL of DNA, 2 mL *DI*, flowrate 5mL/min) in *WE* system. The concentration of DNA

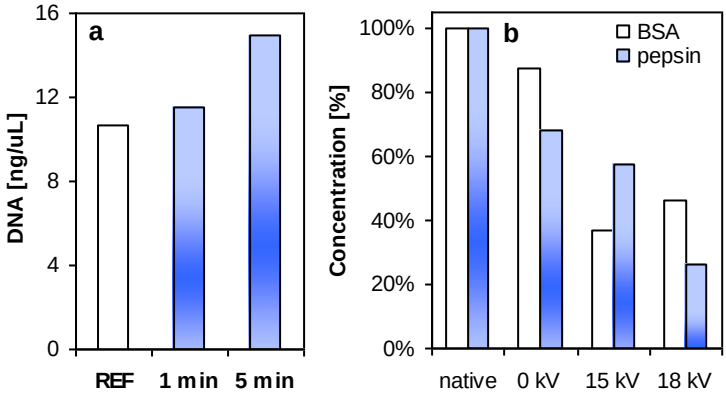

Fig. 5. Concentration of DNA in *WE* system (a) and protein (BSA) and enzyme (pepsin) in *WS* system after direct exposure by *TS* discharge (b).

analysed by UV spectrometry (260 nm) increased in time as a result of DNA fragmentation. Similar characteristics were also obtained for different initial concentrations of DNA and applied voltages. Likewise DNA, solutions of BSA and pepsin were exposed to *TS* discharge of different power and treatment time. Their concentration was monitored by UV (268 nm) and fluorescence (λ_{exc} 285 nm, λ_{em} 340 nm) spectrometry. **Fig. 5b** shows the decrease of their concentration (1mg/mL, 3 mL *DI*, 0.5 mL/min) as the function of the applied voltage in *WS* system. Similar tests performed in WE system showed even stronger effect.

4. Summary

DC transient spark discharge operated in air in contact with water was used for the treatment of cells, DNA, proteins and enzymes. Direct exposure of cells to the discharge in water electrode (*WE)* and water electrospray (*WS*) systems was compared with indirect exposure of cells to *TS* discharge activated gas flow and He pulsed plasma jet. *WE* and *WS* systems showed similar cytotoxicity that increased with treatment time and discharge power. The maximal cytotoxicity of 93.5% was observed for 20 min treatment time and 4 kHz. The results also demonstrated apoptotic behaviour of cells. Stronger effect of plasma exposure was observed for cancerous than normal cells; and for direct exposure than indirect exposure. The cytotoxicity of the plasma jet was found comparable with indirect *TS* discharge exposure, but smaller compared to the direct exposure of *TS* discharge. The treatment of DNA, protein and enzyme in *DI* solutions showed increase of DNA fragmentation and decrease of concentration of biomolecules with discharge treatment time and power. Thus, the results successfully demonstrated the potential of *TS discharge* as the efficient for bio-medical applications.

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References

[1] Janda M., Machala Z., Niklová A., et al., 2012 Plasma Sources Sci. Technol. 21, 045006
[2] Machala Z., Tarabová B., Hensel K., et al., 2013 Plasma Process. Polym. 10, 649
[3] Topala I., Dumitrascu N., Dimitriu D.G., 2012 IEEE Trans. Plasma Sci. 40, 2811