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Effects induced on the cell membrane of *Escherichia coli* by the cold air plasma and the PAW

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Cold air plasmas in direct contact with liquids and air plasma activated water (PAW) and liquids are nowadays of the great interest, since they induce chemical changes via formation of reactive oxygen and nitrogen species (RONS) in the liquid phase. Thanks to the synergistic effects of the plasma agents (electric field, electrons and ions, UV radiation, RONS) and the induced chemical changes in the liquid, cold air plasmas and PAW are known for their bactericidal, cytotoxic, and cell growth stimulation effects [1-2].

We investigated the effects of the direct cold air plasma treatment and indirect treatment by PAW on the bacterial cell membranes (viability and membrane damage by electric field and RONS). Aquaeous solutions (water, phosphate buffer or saline) or bacterial suspensions containing planktonic *E. coli* ATCC 25922 with different buffering capacities and pH were electrosprayed directly through the air transient spark (TS) discharge [3]. Induced chemical changes were examined by the detection of pH changes and RONS (H_2O_2, NO_2^-/NO_3^- and ONOOH/$ONOO^-$) by colorimetric or fluorescent based assays. The direct treatment of bacteria in water showed up to 7 log reduction in contrast with only up to 2 log reduction in buffered solution. The high efficacy in PAW is due to the synergistic effect of acidification (pH~3) and formation of cytotoxic radicals $OH^{\cdot}$, $NO^{\cdot}$ and $NO_2^{\cdot}$; either by the peroxynitrite decay or acidified decay of nitrites. Furthermore, the cell wall and membrane targeted by these radicals and electrically charged particles resulted in the membrane damage (lesions formation, loss of fluidity, increased permeability to H^+, etc.) [4]. We detected an increased number of sublethally injured cell on the selective agar (damage of outer and inner membrane) and oxidative damage of cell membranes in the direct treated PAW due to the lipoperoxidation by RONS. We also attempted to detect the cell membrane electroporation due to the TS-induced electric field by using propidium iodide or DAPI fluorescent staining. In addition, the indirect treatment by PAW (10 min incubation of bacteria) resulted in ~ 4 log reduction. The bactericidal effect of PAW is due to the post-discharge reactions, especially formation of peroxynitrites (from H_2O_2 and NO_2^-) and their fast decay in acidic pH. The bactericidal properties predetermine the PAW as a cheap antibacterial medium. The possibility of its long-term storage by deep freezing was tested.

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