Characterization of bacterial and bio-macromolecule damage by (V)UV and particle channels of X-microscale atmospheric pressure plasma jet (X-µAPPJ)

J.-W. Lackmann[1], S. Schneider[2], F. Narberhaus[1], J. Benedikt[2], J. Bandow[1]
[1]Microbial Biology, Faculty of Biology and Biotechnology, Ruhr-Universität Bochum, Germany
[2]Group Coupled Plasma-Solid State Systems, Faculty of Physics and Astronomy, Ruhr-Universität Bochum, Germany
e-mail: julia.bandow@rub.de

Résumé

Atmospheric pressure plasma jets effectively inactivating bacteria on surfaces including infected tissues. This is due to the combined effects of (V)UV radiation, reactive oxygen and nitrogen species, ions, or high electric fields. Here we present how (V)UV radiation and reactive radical species produced by a micro scale atmospheric pressure plasma jet can be separated by a lateral He flow steering the heavy particles inside the effluent to facilitate the study of mechanisms leading to bacterial cell death. The new jet geometry is called X-jet. The densities of reactive species can be compared to the well characterized geometry of the µAPPJ [1]. We show that heavy particles are steered away by the additional He flow effectively by comparing etching of a-C:H film and inactivation of vegetative *Escherichia coli* and *Bacillus subtilis* cells after exposure to the (V)UV or particles channel. Additionally, by tuning the jet parameters we can select conditions in which either O radicals or O_3 ozone molecules are the dominant species reacting with the biological sample. The results of treatment of bio-macromolecules will be presented here. DNA, RNA and proteins are all damaged by plasma treatment.

Introduction

Atmospheric pressure plasmas are known to be capable of inactivating bacteria. The inactivation is, however, usually only qualitatively studied and the exact inactivation mechanisms and the role of different reactive species (oxygen radicals, metastables, UV photons, ions) and possible synergistic mechanisms among them are not well understood. We use a radio frequency driven atmospheric pressure plasma jet operated with He gas or He/O_2 gas mixture to treat vegetative *E. coli* and *B. subtilis* cells. *E. coli* is a Gram-negative bacterium that is commonly found in the lower intestine of warm-blooded organisms and *B. subtilis* is a Gram-positive bacterium that serves as a model organism for Gram-positive pathogens. The plasma is generated in a square 30 mm long channel with an area of 1x1 mm^2 formed by two electrodes and two glass plates. This source is very well characterized by laser spectroscopy, optical emission spectroscopy, and mass spectrometry. The fluxes of oxygen atoms, ozone molecules, and UV, and VUV photons are known. It is possible to suppress or enhance the flux of the selected species by proper selection of operating parameters and by modification of the source geometry. Additionally, we have modified the exit nozzle of the jet in such a way that a second flow of He gas, which crosses the plasma effluent, steers heavy particles from the jet axis as shown in Fig. 1. We call

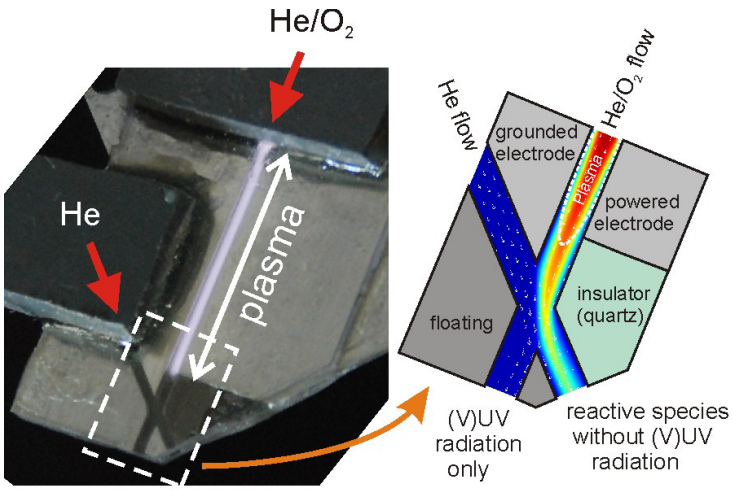

Fig. 1: Left: Photograph of an atmospheric pressure microplasma jet in He/O_2 gas mixture with an additional He flow crossing the plasma effluent. The additional helium flow steers the flow of radical species into a side channel. (V)UV radiation propagates along the line-of-sight with the plasma. Right: fluid dynamics simulation of the flow in the X-Jet. Arrows correspond to gas velocity and the color map represents concentration of reactive species (e.g. O atoms; scale increases from blue to red). This simulation illustrates the separation of (V)UV and heavy reactive particles.

this modified jet X-jet. The (V)UV radiation propagating on the line-of-sight is then effectively separated from heavy particles. The effect of these plasma components, (V)UV radiation and reactive heavy particles on the *E. coli* and *B. subtilis* cells can be studied separately. This is demonstrated in Fig. 2, which shows inhibition zones of *B. subtilis* vegetative cells on agar medium produced by the X-jet without and with additional He flow after 1 and 6 minutes of treatment. Without the steering He flow, a typical dose-effect relationship could be observed. Elongating treatment time resulted in increasing inhibition zones. Sample treatment with activated steering flow resulted in two separate inhibition zones. The upper inhibition zone exposed to the effluent of the direct channel was always smaller than the lower inhibition zone exposed to the effluent of the crossed channel. No inhibition zone(s) could be observed after 1 minute of plasma treatment indicating higher efficiency of the combined treatment. The microplasma jet and its X-jet modification are also characterised by means of molecular beam mass spectrometry (MBMS) in similar ways to those described in [1]. Inactivation efficiencies are also compared.

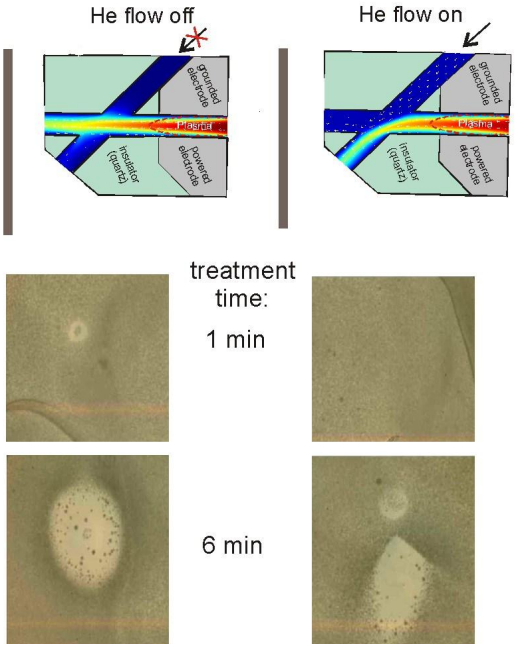

Fig. 2: Inhibition zones after treatment of *B. subtilis* without and with additional He flow. Treatment geometry as indicated at the top.

Besides treating whole bacteria, plasma impact on bio-macromolecules is investigated. DNA-, RNA-, and Protein damages might play an important role in bacterial inactivation. The different damaging effects between (V)UV and particles are investigated and compared thanks to the X-jet. Plasma not only induces single and double strand breaks into DNA, it is also capable of cross-linking several DNA fragments into plasmid dimers or multimers (Fig. 3a), as well as inducing different kinds of nucleotide specific alterations. In contrast, RNA is mostly damaged via the induction of single strand breaks or by plasma based etching (Fig. 3b). The latter also applies to plasma induced protein damage. Nucleotides were dried and treated on glass slides, washed and analyzed via agarose gel electrophoresis. Etching experiments with BSA were observed using a laser scanning microscope and compared to the typical etching model of an a-C-H coating to differentiate between etching impact on a biological sample compared to non-biological materials.

With the help of the X-jet, we are able for the first time to analyse damaging effects of different effluent components under *in vivo* and *in vitro* conditions. We will further investigate plasma impact on bio-macromolecules, as these have a huge impact on clinical and industrial applications and will further deepen our understanding about the basic bacterial inactivation mechanisms.

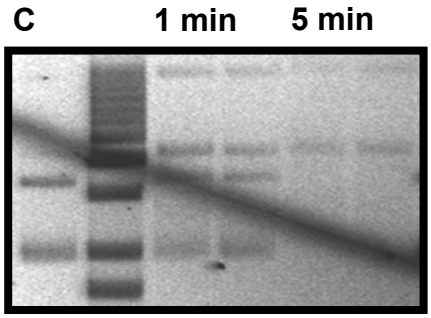

Fig. 3a: Dried pUC18 plasmid DNA treated with undivided effluent. Control (C) shows supercoiled and relaxed (lower and upper band respectively) plasmid form. After plasma treatment, multimeric and dimeric plasmid forms occur.

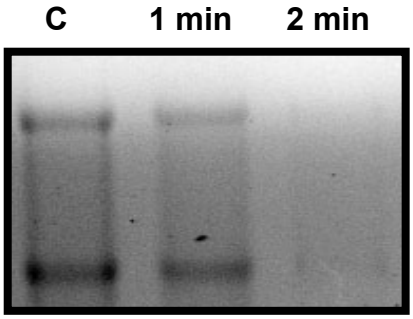

Fig. 3b: Total *E. coli* RNA treated with undivided effluent. Loss of intensity of distinct 23S und 16S rRNA (upper, lower band respectively) indicates etching and

References

[1] D. Ellerweg, J. Benedikt, A. von Keudell, N. Knake, V. Schulz-von der Gathen, New J. Phys. **12** (2010) 013021.