Max-Planck-Institut für extraterrestrische Physik

Inactivation of Bacteria in Solution by Atmospheric Pressure Plasma: Density Effects

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Purpose

Bactericidal, fungicidal, virucidal and sporicidal effects of cold atmosphere pressure plasmas were under intensive investigation in the past few years. Reactive species (besides charged particles) produced by the plasma are believed to play a crucial role in this. Most of the recent studies focused on occurring reactive oxygen species (ROS) during the plasma application and on oxidative stress on microorganisms. The generation of ROS and the resulting lipid peroxidation is believed to cause a loss in membrane integrity. The purpose of this study is to take into account not only the involvement of ROS but also reactive nitrogen species (RNS). Additionally influences of initial cell densities and different plasma treatment times were taken into consideration. *E.coli* were treated in solution for up to 8 minutes with initial cell densities between 10^2 and 10^8 cells per 20 µl with a plasma device, which uses the Surface Micro Discharge (SMD) technology and the surrounding air for plasma production. The products of a few chemical reactions between the reactive species produced by the plasma and the liquid (with and without bacteria) were examined and analyzed. During the first 2 minutes of plasma application hydrogen peroxide and reaction products of NO rapidly occurred. The evidence of NO uptake by bacteria and further reference experiments with hydrogen peroxide clearly showed that the bactericidal properties of plasmas are a combination of oxidative and nitrosative effects. To evaluate the influence of dissolved peroxides and products of reactions with NO in liquids respectively reference experiments with H_2O_2 and a chemical NO donor were performed.

Experimental Design

Treatments of all bacteria were performed in Tris buffered Saline (TBS) and on agar plates. Several plasma treatment times and standard assays following plasma treatment were used to assess dissolved species, the density effect and the time dependency.

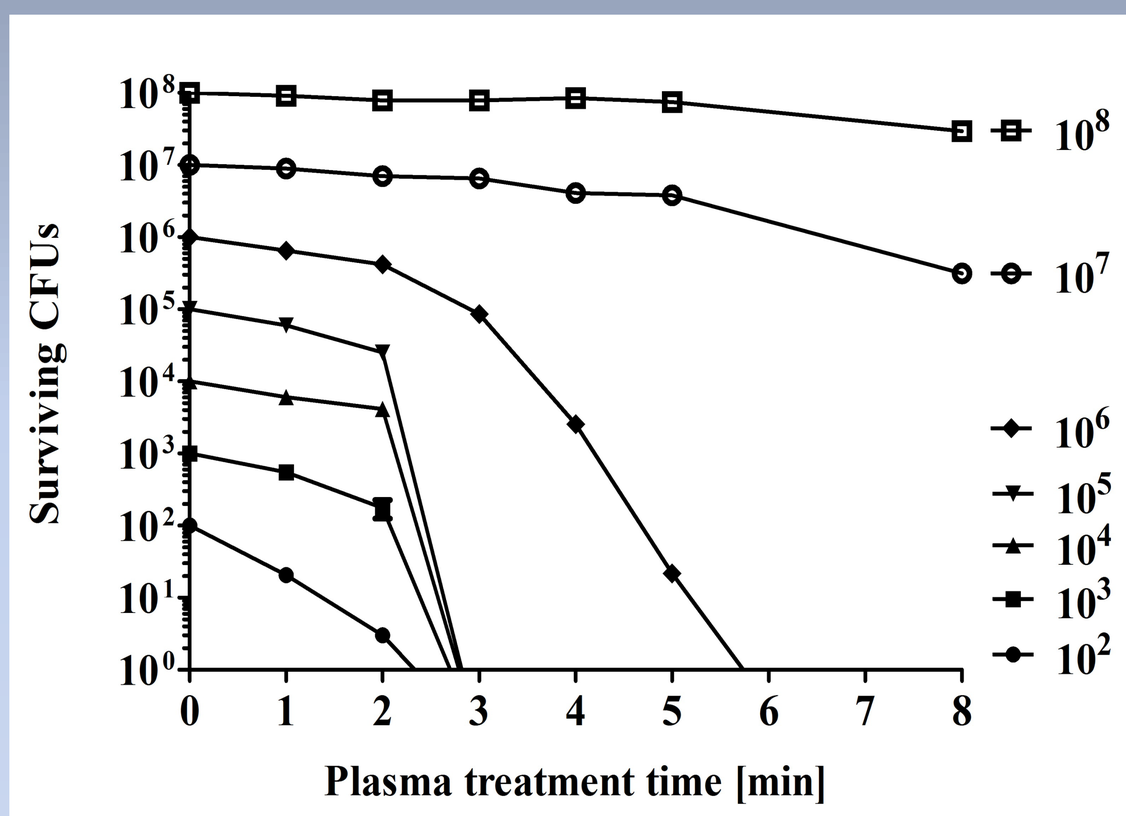

Figure 1:
Kinetic inactivation curve of 10^2 – 10^8 E.coli cells in 20 µl TBS. The bacteria were treated for 1, 2, 3, 4, 5 and 8 minutes with CAP. A 5 log reduction was achieved for 3 minutes of CAP application for initial cell densities of 10^5 per 20 µl or lower. For cell densities of 10^7/20 µl and higher almost no reduction was achieved for CAP treatment times of up to 8 minutes.

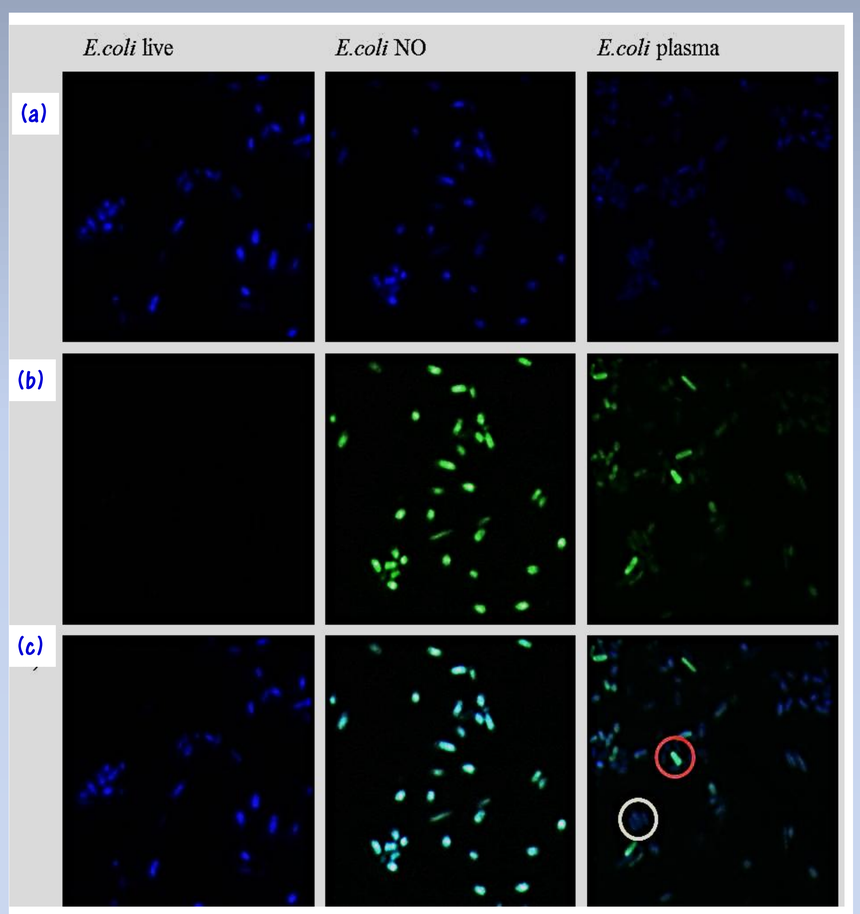

Figure 4:
Living E.coli cells (E.coli live), E.coli cells treated with a chemical NO donor (E.coli NO) and E.coli cells, treated with plasma for 3 minutes (E.coli plasma) (a) Channel 1: DAPI staining; (b) Channel 2: DAF-2 staining; (c) Channel 1 + 2 merged.
All bacteria are stained by DAPI (blue), bacteria that took up NO are stained by DAF-2 (green). Plasma treated bacteria fluoresce in green like those who were treated with an chemical NO donor and in contrast to untreated, living E.coli cells. This means they took up NO during or as a consequence of the treatment. The merged image of plasma treated E.coli shows clearly that the uptake of NO is not regularly. The red circle shows a bacterium which took up much more NO than those in the yellow circle.

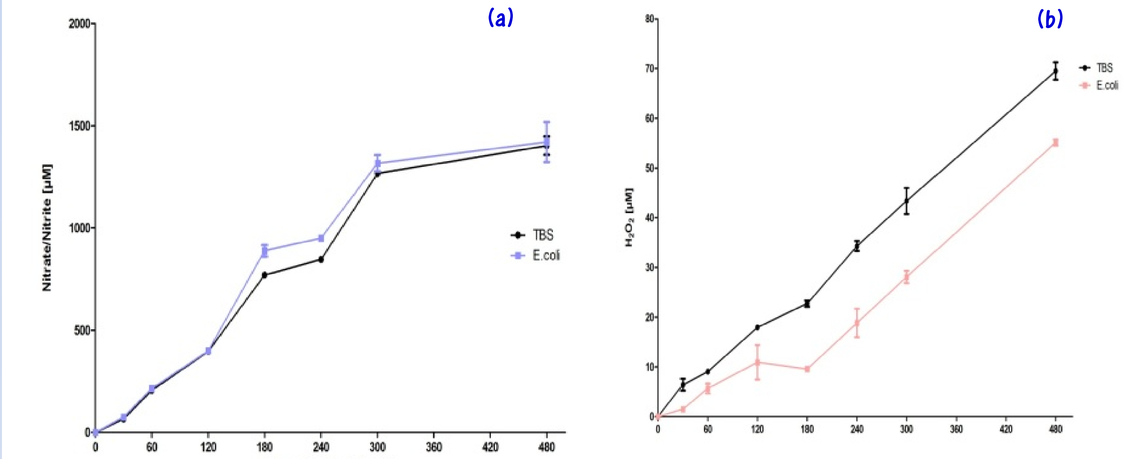

Figure 2:
Solved reactive species in TBS after plasma application.
(a) Nitrates and (b) peroxides

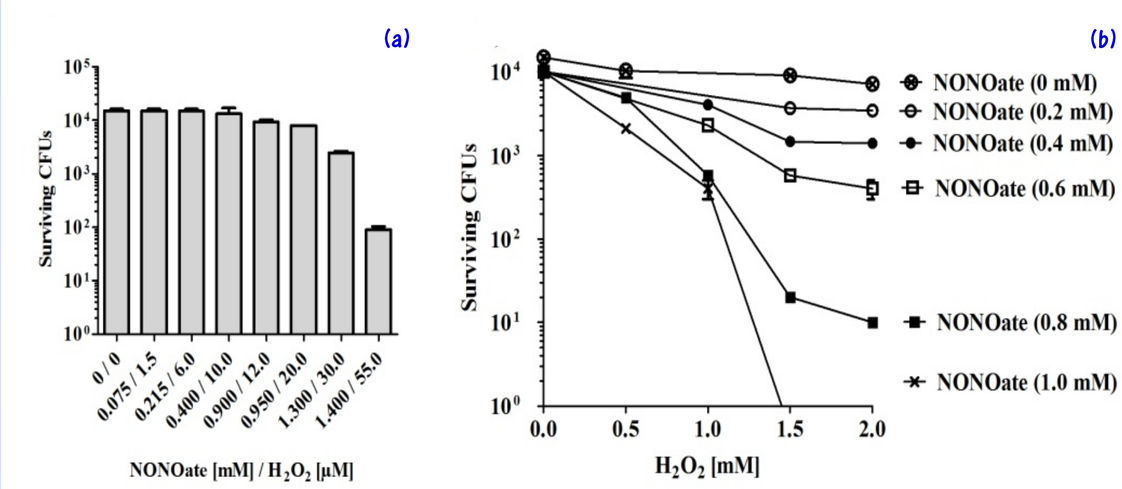

Figure 3:
Reference experiment with NONOate and H_2O_2. The concentrations correspond to those after 1, 2, 3, 4, 5, 6 and 8 minutes of plasma application. b) Reference experiments with higher concentrations of NONOate and H_2O_2.

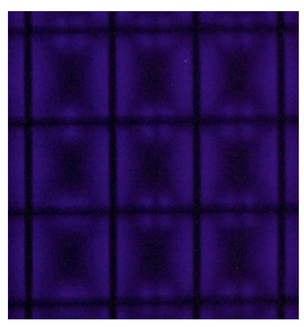

Figure 5:
The Surface Micro Discharge (SMD) Technology: the discharge is produced in the squares of the mesh electrode. The black lines in the figure are the wires of the mesh electrode.

Results and Conclusion

Cold atmosphere pressure plasma (CAP) is able to easily kill pathogens like EHEC and MRSA within seconds. Our results show evidence of not only reactive oxygen but also nitrogen species being involved in the cascade of the bactericidal action of CAP. Reactive oxygen and nitrogen species are also weapons of the immune system against microorganisms. It is therefore fair to assume that CAP is not only a very effective tool for disinfection but also highly tolerated by mammalian organisms which makes CAP applicable in a broad field of medicine, healthcare and hygiene.

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