Max-Planck-Institut für extraterrestrische Physik

Inactivation of different microorganisms including EHEC and MRSA by non-thermal atmospheric plasma devices

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Purpose

Cold atmosphere pressure plasma (CAP) is one of the most promising prospective tools for the prevention of infectious diseases and nosocomial infections. It easily kills a wide range of microorganisms like bacteria, fungi, viruses and spores. It was therefore necessary to find out if CAP is also able to inactivate bacteria resistant to antibiotics like *multi resistant Staphylococcus aureus* (MRSA) and *verotoxin-producing Escherichia coli* (EHEC), which can cause severe diseases.

The bactericidal mechanism by CAP is not clarified yet. However, it is believed that there are several processes which lead to inactivation of microorganisms. There is a common consensus that reactive species play a major role in this. Recent results show that reactive oxygen species cause peroxide emergence which finally leads to lipid peroxidation and other oxidative damages in the cell wall of the microorganisms. As the plasma of the device used in our studies is created in air not only reactive oxygen but also nitrogen species occur.
Aim of this study was to determine efficacy and influences of CAP.

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 probability to build up resistance against plasma and the efficacy against MRSA and EHEC.

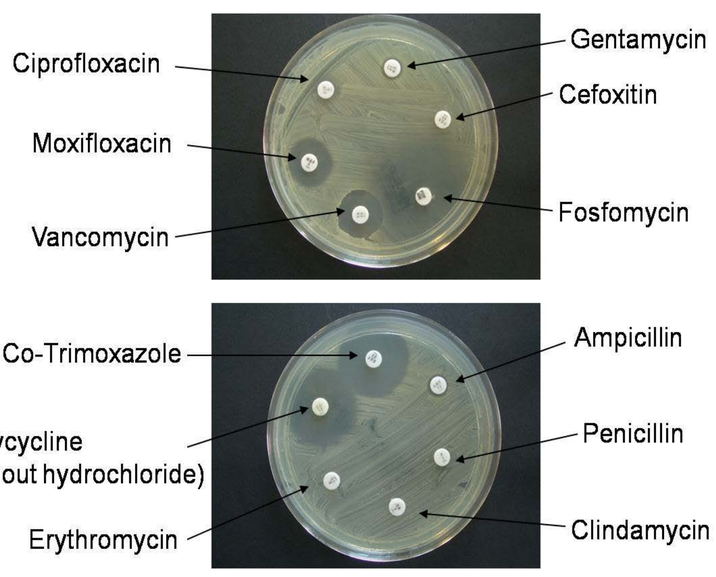

Figure 1:
Antibiotic resistance of the used MRSA strain. The MRSA strain shows resistance against Penicillin, Ampicillin and Cefoxitin (all Beta-Lactam antibiotics) and additionally to Gentamicin (Aminoglycosides), Erythromycin (Macrolides), Doxycycline (Tetracycline's), Ciprofloxacin (Crinolines) and Clindamycin (Lincosamines).

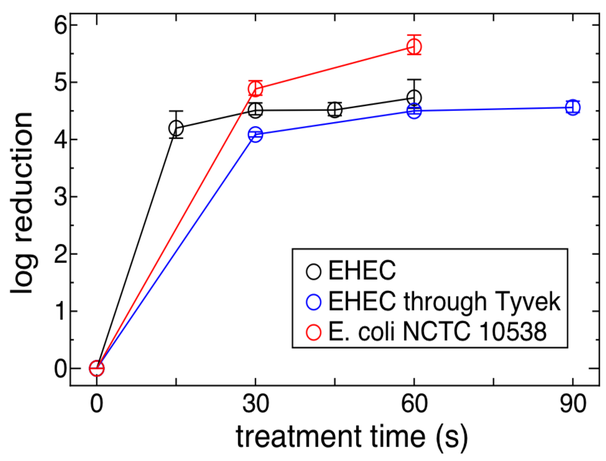

Figure 2:
Kinetic killing curve of EHEC treated with CAP. A log 5 reduction was achieved within 30 seconds.

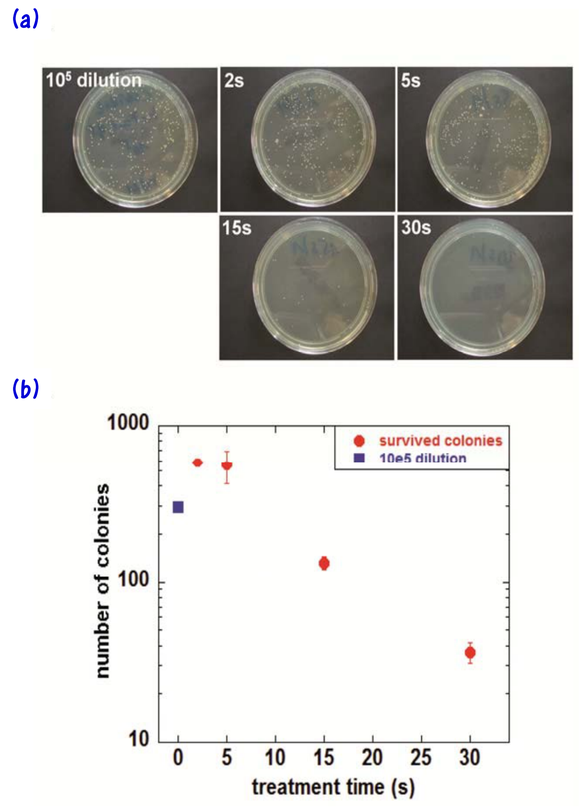

Figure 4:
Experimental results of the performance tests with MRSA.
(a) Photos of agar plates containing MRSA colonies after an incubation time of 18 hours at 35°C. The left agar plate contains 200 µl of a 10^5 dilution of the original bacteria suspension and therefore functions as a control. The other four agar plates were treated by the plasma flow from the plasma dispenser for 2 s, 5 s, 15 s and 30 s.
(b) Number of survived bacteria (i.e. number of MRSA colonies) for different plasma treatment times (red data points). For comparison, the 10^5 diluted control sample is shown additionally (blue data point).
In less than 10 s the plasma dispenser reduces MRSA by a factor larger than 10^5.

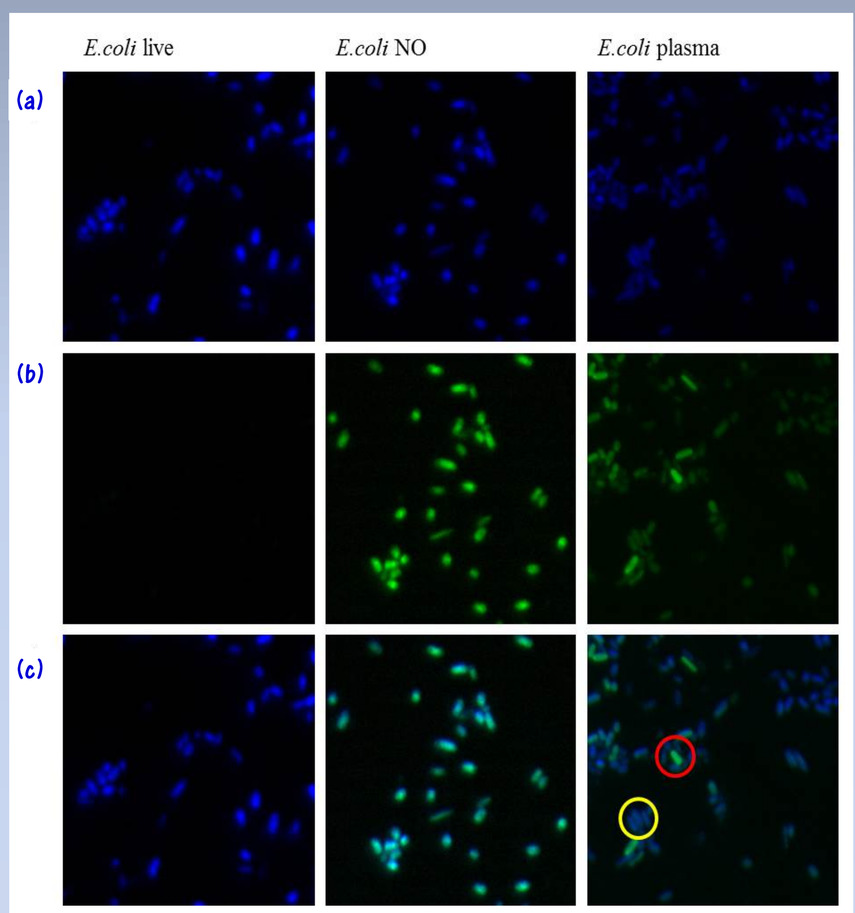

Figure 5:
Living E.coli cells (E.coli live), E.coli cells treated with a chemical NO donor (E.coli NO) and E.coli cells, treated with plasma for 3 minutes (E.coli plasma)
(a) Channel 1: DAPI staining; (b) Channel 2: DAF-2 staining; (c) Channel 1 + 2 merged.
All bacteria are stained by DAPI (blue), bacteria 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.

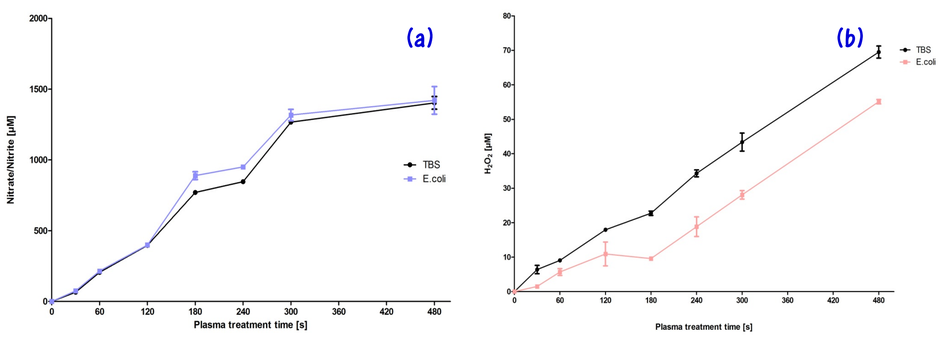

Figure 3:
Solved reactive species in TBS after plasma application.
(a) Nitrates and (b) peroxides

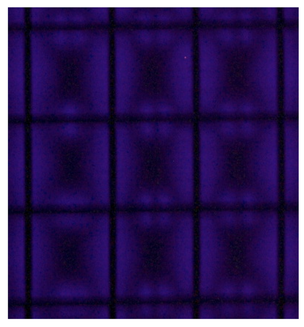

Figure 6:
The Surface Micro Discharge (SMD) Technology: the discharge is produced in the squares of the mesh electrode. The black lines in the figure are the wires of the mesh electrode.

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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