Effects of Plasma Generated Ions on Bacteria

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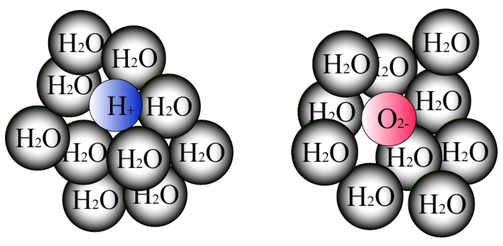
Introduction

In 2000, the Sharp Inc. introduced the "PlasmaCluster Ions® (PCI)" air purification technology. It is based on the emission of plasma generated ions surrounded by water molecules.



Aims of the study

- Ion emission verification
- Effects on various gram-positive microorganisms
- Time-dependency and irreversibility
- Spatial and kinetic characteristics of the emitted clusters
- Biomolecular targets of PCIs in microbial cells.

Materials and methods

Bacterial strains used

Staphylococcus chromogenes ATCC 43764
Enterococcus malodoratus ATCC 43197
Sarcina flava (Micrococcus luteus), ATCC 4698
Micrococcus roseus (Kocuria rosea) ATCC 186
Bacillus subtilis subsp. *subtilis* ATCC 6051

Evaluation of bacterial viability

Colony-forming units (CFU) count
Differential fluorescent staining

Plasma cluster ion generation and detection

Plasmacluster® Ion Generator provided by Sharp Corp.
Ion detector „IST-801A" and Run-Time Engine 6.02
by National Instruments

Time-course of PCI effects

Variants differed only in the duration of exposure to PCIs
Samples taken at 0, 30, 60, 120, 180, 240 and 480 min
After 48 h of incubation the colonies on agar were counted.

Spatial distribution of emitted PCIs

Estimation of bacterial growth inhibition rate and, second, using the so called "substrate film" method.
In a separate series of experiments, the ability of plasma generated ions to pass shadowed areas was checked as shown in figure 1.

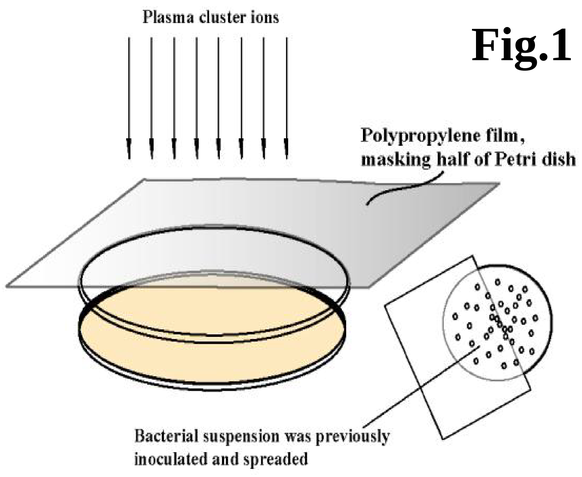


Fig.1

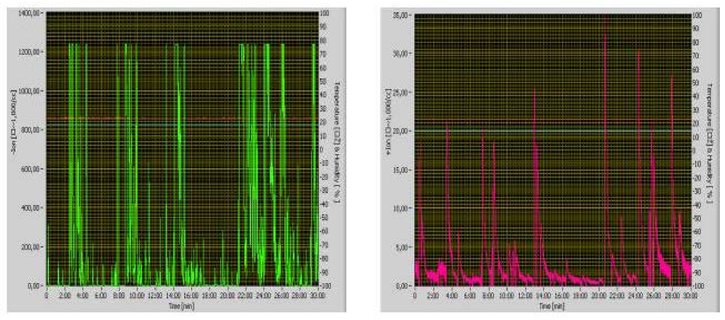
Analysis of bacterial proteins

Changes in protein composition of microbes were detected by SDS PAGE.

Results and discussion

Ion emission:

Figure 2. shows a typical plot of positive as well as negative ion emissions over time (30 minutes).

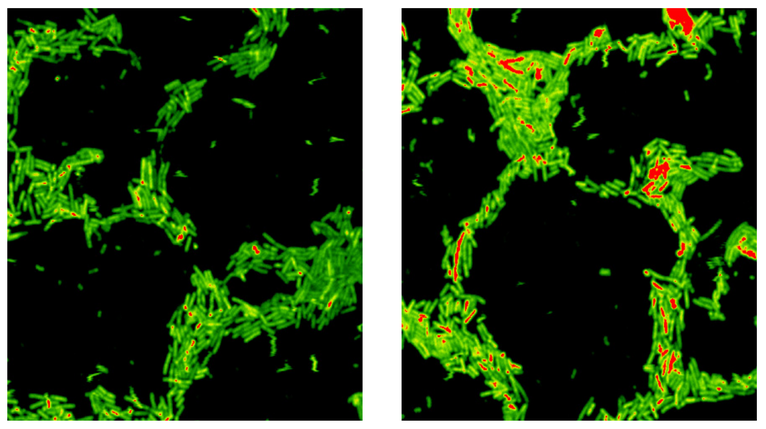


Negative Positive

Fig.2

Morphology and viability:

After treatment with plasma-generated cluster ions for 6-8 hours profound changes in the colony morphology was observed. Colonies lost their typical round and smooth appearance and a lot of radial crackles appeared. Figure 3 shows fluorescent microscope images of Bacillus subtilis taken before (control) and after six hours of PCI treatment. A very high percentage of damaged cells (red and yellow) became visible after treatment.



Control **Fig.3** 6 hours PCI

Time-dependency and irreversibility:

Figure 4 illustrates the effect of cluster ions on the proliferation of bacteria as compared to control. The exposure to PCIs caused significant and progressive reductions in the numbers of viable cells with exposure time. Three hours of incubation resulted in almost full inhibition of the growth.

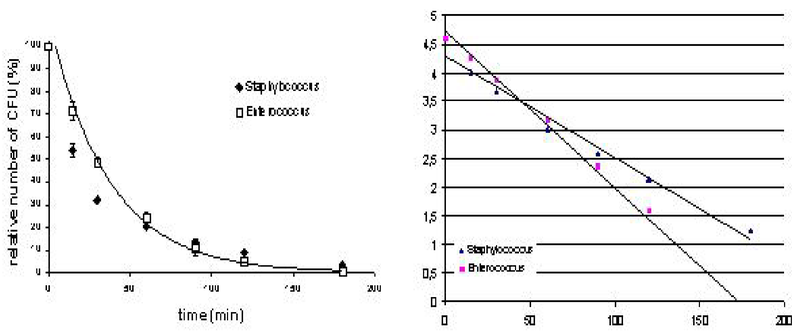


Fig.4

Spatial distribution, life time, propagation:

First, Petri dishes were inoculated with bacteries. Then they were mounted along all walls of a test chamber (21*14*14cm^3) containing an Sharp ion generator. After 3 hours of PCI exposure, the Petri dishes were taken to the incubator and bacteries were cultured for 48h. Afterwards the colonies were counted. The experiments showed that most of the PCIs were emitted into the front-downward direction (Figure 5). Thus, the ion clusters must be pretty "heavy" particles with an initial momentum of movement and a relatively short lifetime.

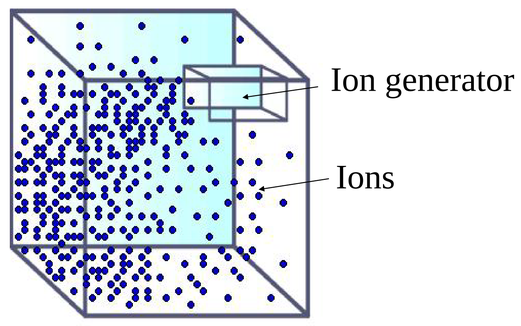


Fig.5

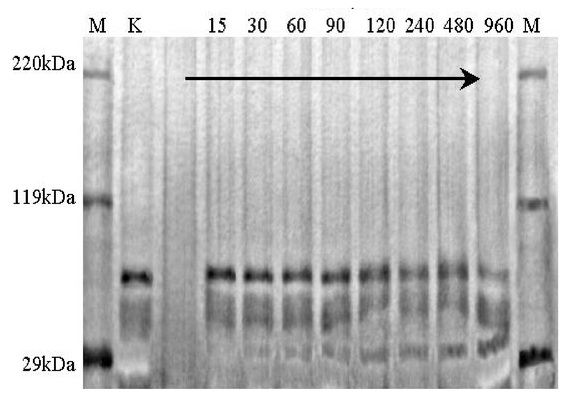


Fig.6

Influence of PCI on bacterial proteins:

Cellular proteins of Enterococcus malodoratus separated by SDS-PAGE, are shown in Figure 6 for different times of PCI exposure. The 93 kDa band fades out with increasing exposure time; whereas the 34 kDa band became more prominent showing the protein degradation caused by PCIs.

Fig.7

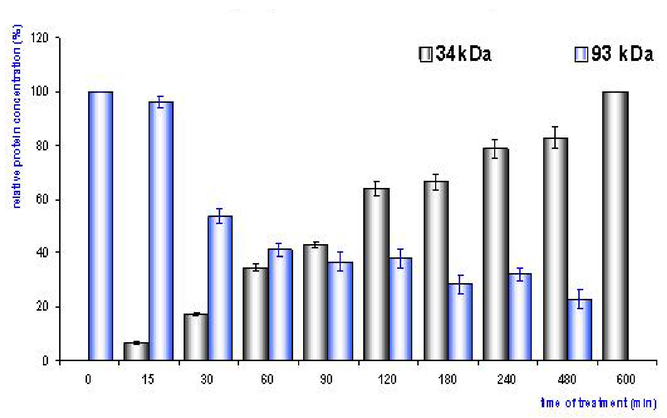


Figure 7 shows the relative protein content of two bands, 34 kDa (grey) and 93 kDa (blue), respectively, during the PCI exposure.

Summary and Conclusions

- PCIs were clearly effective in terms of their antibacterial effects with the strains tested.
- This efficacy increased with the time the bacteries were exposed to PCIs.
- The bactericidal action has proved to be irreversible.
- PCIs were significantly less effective in shadowed areas.
- PCI exposure caused multiple protein damages as observed in SDS PAGE studies.
- There was no single but multiple molecular mechanism causing the bacterial death.