GASEOUS PLASMA FOR SURFACE STERILIZATION

A. POIATA[1], I. MOTRESCU[2], A. NASTUTA, D. CREANGA, G. POPA,
[1]University *Grigore T. Popa*, Faculty of Pharmacy, Iasi, Romania,
[2]University *Ion Ionescu dela Brad*, Biophysics Department, Iasi, Romania,
[3]University *Alexandru Ioan Cuza*, Faculty of Physics, Iasi, Romania

Rezumat

Plasma gazoasă pentru sterilizarea suprafeţelor

Interacţiunea plasmei gazoase cu culturi bacteriene a fost studiată ca aplicaţie practică a plasmei reci la sterilizarea obiectelor mici pentru utilizare medicală. Zonele de inhibiţie a creşterii microbiene au fost măsurate în jurul spotului de impact cu jetul de plasmă pentru un jet de 3.5 cm lungime şi pentru unul de 2.5 cm, pentru acelaşi timp de expunere de 50 secunde. Efectul evident de distrugere a bacteriilor recomandă această metodă pentru sterilizarea unor ustensile medicale ce sunt sensibile la temperatură sau la reactivi chimici, ea fiind de asemenea susţinută de relativa accesibilitate şi costurile mici.

Cuvinte-cheie*: culturi bacteriene, plasmă rece, sterilizarea suprafeţelor*

Резюме

Газовая плазма для стерилизации поверхностей

Взаимодействие газообразной плазмы с культурами бактерий было исследовано с точки зрения практического применения холодной плазмы для стерилизации малых предметов медицинского пользования. Зоны подавления микробного роста были измерены вокруг места воздействия струй плазмы для струи с длиной 3,5 см, а также с длиной 2,5 см при одинаковом времени воздействия равной 35 сек. Благодаря очевидному эффекту уничтожения бактерий, этот метод рекомендуется использовать для стерилизации некоторых медицинских принадлежностей, чувствительных к температуре или химическим реактивам, метод будучи удобным также благодаря относительной доступности и малой стоимости.

Ключевые слова*: бактериальная нагрузка, холодная плазма, стерилизация поверхностей*

Introduction

Various attempts of searching for efficient factors to kill bacteria at room temperature were reported. Mainly UV rays were used for decades to sterilize laboratory surfaces. Conventional methods such as heat, irradiation, or chemical gases, can alter material features: surface and volume morphology, molecular weight and others [1]. In contrast cold atmospheric plasma preserves such material characteristics especially important for polymers that are so sensitive to high temperature and chemicals [2, 3].

Cold atmospheric plasma is a physical system composed by weakly ionized gases generated by means of an electric discharge between two electrodes. Small ions resulted from atmospheric gases together with free electrons are conducted through the electric field between the electrodes and interact with any object placed between them. The main effects are further ionization, dissociation and excitation of atoms and molecules from the space volume between the discharge electrodes; thus living cells such as those of microorganisms could be seriously damaged as long as physical changes results very quickly in biochemical perturbation. Spores of *Bacillus subtilis* were inactivated with cold atmospheric plasma as reported in [4, 5]. Decolonization of *Escherichia coli* and *Staphyloccocus aureus* with cold plasma treatment was successfully performed also [6] while remarkable progress in *Candida albicans* inactivation was reported too [7]. We also made some attempts to get microorganism killing with plasma jet as shown in [8, 9] for 25, 50, 75 and 100 s.

In this article specific results are presented for a new exposure time that we have tested by using dielectric barrier plasma discharge.

Materials and methods

Microorganisms. Gram positive and Gram negative strains were chosen: *Sarcina lutea* ATCC (American Type Culture Collection) 9341, *Bacillus cereus* ATCC 14579, *Pseudomonas aeruginosa* ATCC 9027 and *Escherichia coli* ATCC 35218 – all acquired from standard collection ATCC. The standard microbial vials were preserved in lyophilized form until the experiment took place. Bacterial inoculum were prepared in normal physiological sterile saline to have a density of approximately 3.10^8 cells/ml adjusted through calibration curves. Small volumes of 0.2 ml from each inoculum were seeded in 10 ml Mueller-Hinton (Oxoid) molten agar (pH 6.8). Final agarized cultures were let to grow in 100 mm diameter sterile plastic Petri plates.

Cold plasma yielding and sample treatment. Plasma jet, at atmospheric pressure was yielded by asymmetric dielectric barrier discharge (DBD), using helium at a gas flow of 0.15 l/min; the application of a pulsed voltage of 9 kV – peak to peak value, at 1.6 kHz frequency was carried out, as described elsewhere [10]. The sample exposure was arranged for two distances between the high voltage electrode and the grounded one: 2.5 and respectively 3.5 cm. Every Petri dish with bacteria cultures was inserted into the discharge electrodes on dielectric layer placed over the plane ground electrode. The duration

of every bacterial sample exposure lasted for 35 s with four identical repetitions on every Petri dish for either 2.5 cm or 3.5 cm plasma jet length. All Petri dishes were afterward let to incubation for 24 h at 37.0±0.5°C according to established microbiological protocols [11]. The control samples were Petri dishes inoculated from the same bulk vial but not exposed to plasma treatment. Circular zones of inhibition growth were measured with 1 mm precision ruler. The colony forming units (CFUs) were also counted to assess the efficacy of the microorganism killing.

Statistics. Average values and standard deviations resulted from four repetitions for every exposure arrangement were used for graphical representation.

Results

In *fig. 1* the response of *Escherichia coli* to the action of cold plasma is presented. The four repeated exposures to the plasma jet of 2.5 cm length resulted in quasi-equal circular areas.

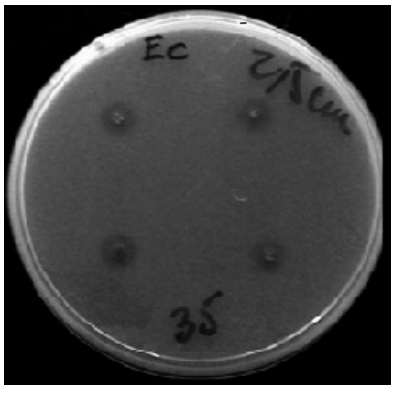

Figure 1. *Escherichia coli response to 2.5 cm plasma jet*

Similar pictures were obtained for the other microorganism strains corresponding to the 2.5 cm distance between the plasma jet edges. In *fig. 2* the results describing the behavior of all four microorganisms following the impact of 2.5 cm plasma jet can be seen.

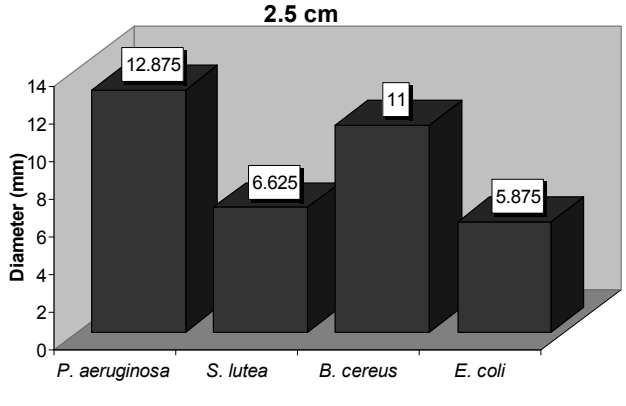

Figure 2. *The diameter of the inactivation areas for 2.5 cm plasma jet*

Maximum sensitivity was found for *Pseudomonas aeruginosa* with average value of the growth inhibition diameter of 12.875 mm, followed by *Bacillus cereus* – with 11 mm average diameter, *Sarcina lutea* – with 6.625 mm and *Escherichia coli* with 5.875 mm. Standard deviation was of about 9 %. Different situation was evidenced for longer plasma jet of 3.5 cm (*fig. 3*). The most peculiar behavior was found for the two Gram positive strains, namely *Sarcina lutea* and *Bacillus cereus* that exhibited no sensitivity to the plasma flow of ions, electrons and ions accompanied by UV photons released through the interaction of accelerated particles with the air and water vapors-as they are at the end of the 3.5 cm tracks between the discharge electrodes. The two Gram negative microorganisms were still sensitive to cold plasma impact evidencing inhibition areas with diameters of 6.25 mm and 4.25 mm respectively (*fig. 3*). The counting of colony forming units within the growth inhibition areas was assessed as ranging between 3% in the case of *E. coli* and 27% in the case of *B. cereus* for 2.5 cm and between 5% in the case of *E. coli* and 32% in the case of *P. aeruginosa* for 3.5 cm. Thus the inactivation efficiency could be assessed, complementary, as ranging between 97% and 73% and respectively between 95% and 68%.

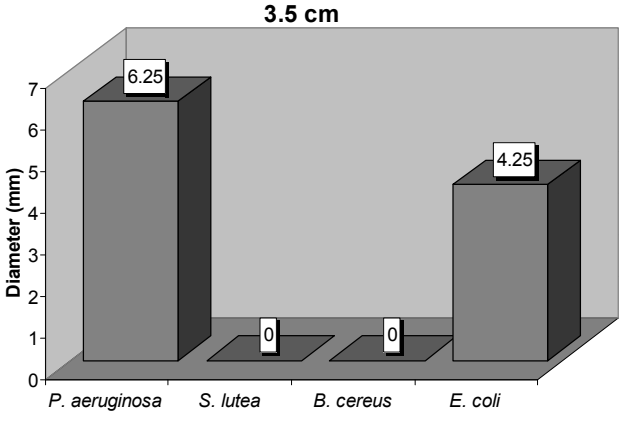

Figure 3. *Inactivation area diameters for 3.5 cm plasma jet*

Smaller inhibition zones for 3.5 cm compared to 2.5 cm could be related to diminished physical impact of corpuscular radiation (ions, electrons, free radicals) because of atmospheric air higher attenuation. Higher sensitivity of Gram negative germs for 3.5 cm plasma jet could be assigned to higher content of UV generated ozone. We assume that accelerated electrons hit oxygen and nitrogen molecules favoring reactive species formation and UV and visible photons release with further ozone generation [12]. This needs to be correlated with relative higher sensitivity to ozone of Gram negative bacteria, because of their higher content of proteins in the cellular wall [13]. The atmospheric plasma

sensitivity evidenced in this experiment for the Gram negative germs – otherwise known for their complex cellular wall and higher resistance, is consistent with the fact that the physical treatment based on low temperature plasma jet could be still efficient for the inactivation of those bacteria that usually can be killed with certain difficulties by purely chemical treatments (drugs or disinfection chemicals).

Conclusion

Cold plasma discharge in specific laboratory arrangement and with optimized physical parameters was found efficient in microorganism inactivation. Gram positive bacteria were more sensitive for 2.5 cm plasma jet compared to Gram negative bacteria that were more sensitive to 3.5 cm plasma jet.

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