Bacterial biofilm inactivation by gas discharge plasma: overview and future perspectives

G. Brelles-Mariño
Biological Sciences Department, California State Polytechnic University, Pomona, USA
CINDEFI, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina
e-mail: gbrelles@csupomona.edu, gbrelles@biotec.org.ar

Résumé

Bacterial biofilms are more resilient to standard killing methods than free-living bacteria. Our results show that gas discharge plasmas are a novel alternative to inactivate/sterilze biofilms. However, viability experiments have to be carried out before drawing the conclusion that plasma kills cells based solely on their culturability. Non culturable cells retain viability and pathogenicity after short exposures to plasma.

Overview

Most studies dealing with growth and physiology of bacteria have been carried out using free living (planktonic) cells. These studies have provided extensive information regarding the basic molecular mechanisms controlling the growth of individual bacteria. However, most bacteria live primarily in communities referred to as biofilms. Biofilms are microbial communities embedded in an exopolysaccharidic matrix and responsible for undesirable effects including disease, biofouling, pipe plugging, corrosion, dental plaque, and prosthetic device contamination, just to mention a few. Cooperative interactions among members of the biofilm make conventional methods of controlling microbial growth often ineffective. Therefore, there is a need to develop novel inactivation/sterilization tools and the use of gas discharge plasmas represents an alternative to traditional methods (revised in [6]).

My laboratory first studied biofilms produced by *Chromobacterium violaceum*, a bacterium present in soil and water. Biofilms were subjected to plasma for different exposure times and 99.6% of culturable cells were inactivated after a 5-minute treatment [1-5]. Atomic force microscopy (AFM) images revealed sequential changes in cell morphology occurring during plasma treatment [4]. However, physiological and metabolic determinations, and fluorescence microscopy showed that non-culturable cells were still alive after short plasma exposure times. These results indicated that viability experiments are indispensable before drawing the conclusion that plasma kills cells based solely on culturability [5].

We are presently studying plasma-mediated inactivation of *Pseudomonas aeruginosa* biofilms. This bacterium is an opportunistic pathogen that preys on victims with compromised immune systems, patients on respirators, and causes infections of burned tissue and colonization of catheters and medical devices. We have recently reported the effect of plasma on *P. aeruginosa* strain PAO1 biofilms grown on borosilicate coupons [7]. We are now looking at the effects of gas discharge plasma on 1, 3, and 7-day-old biofilms of *P. aeruginosa* grown on polycarbonate, stainless steel, or borosilicate coupons in a CDC biofilm reactor (BioSurface Tech, MT). An atmospheric pressure plasma jet is generated with an Atomflo^{TM} 300 reactor (Sur*fx* Tech. CA) using a mixture of He and N_2 gases. Biofilms are exposed to plasma for various exposure times and processed to determine CFUs/mL after incubation. Results indicate nearly 100% of biofilm inactivation after 5-minutes of plasma exposure. The inactivation kinetics are similar for 1, 3, and 7 day-old biofilms and show a rapid decline in the number of surviving cells followed by a much slower decline. No differences were observed for the three materials used. The inactivation kinetics is similar to the one obtained for *C. violaceum* biofilms [5], suggesting that the method is useful regardless the type of biofilm treated. AFM images show changes in cell morphology and biofilm structure for various plasma exposure times. Micromechanical properties of biofilms are studied through force versus distance curves [7].

One of the issues of more concern regarding work dealing with plasma-assisted cell inactivation is that, in most of the cases, the lethality of plasma is assessed based on the number of cells that can be cultured after the treatment. However, bacterial cells can respond to stress by entering a viable-but-non-culturable (VBNC) state. The fact of not being able to culture bacteria after plasma treatment cannot be considered as a clear-cut indication that the cells are dead. The VBNC state is a survival mechanism of bacteria facing environmental stress conditions. Bacteria enter into this dormant state in response to one or more

environmental stresses, which might otherwise be ultimately lethal to the cell. When cells are VBNC they are unable to produce colonies in an agarized medium but they are still alive and may retain pathogenicity. To test this hypothesis for *P. aeruginosa* biofilms, we developed a virulence assay using lettuce plants. The central vein of healthy lettuce leaves was injected with a bacterial suspension exposed to plasma for different exposure times. A non-exposed control was also included. Results show that *P. aeruginosa* cells that are non culturable after a short exposure to plasma, are still pathogenic.

Our results clearly show that bacterial biofilms can be inactivated by using gas discharge plasma. The architecture and the stability of the biofilm, together with cell culturabilty, are impacted by the plasma treatment. These results are evidence of the potential for plasma as an alternative sterilization method against biofilms. However, viability experiments should always be carried out before drawing the conclusion that plasma is useful to kill cells based solely on measurement of culturable cells. Research is being carried out in our laboratories to try to better understand the mechanism leading to cell inactivation. We expect that our study will provide the fundamental understanding of plasma-assisted biofilm inactivation and its mechanisms and build the basis for the future development of the technology.

Future perspectives

The results discussed are an evidence of the potential of gas discharge plasma to inactivate bacterial biofilms. The technology is clean and reported to be safe in medical settings for both the patient and the operator, although more research is needed to test the safety of the procedure. In any case, care has to be taken before drawing conclusions about the complete removal of biofilm-forming cells. A single cell detached from a biofilm is able to adhere to a surface and trigger the development of a new biofilm. Therefore, if the technology is to be applied to pathogenic organisms in health-related settings, this aspect is particularly crucial to prevent recontamination of surfaces. This problem can be easily solved if viability experiments are carried out at the same time.

More research has to be carried out in order to determine plasma parameters and conditions that would completely sterilize biofilms. The technology is still somehow expensive compared to other sterilization methods. However, as most of those methods are ineffective towards biofilms or cannot be applied to all circumstances, the use of plasma still offers many promising opportunities for application. So far, results have been obtained with some model organisms but most of the naturally occurring biofilms are mixed populations. In years to come, we expect to see more research on mixed biofilms and the development of plasma applicators with different geometries.

References

[1] G. Brelles-Mariño, J.C. Joaquin, J.D. Bray, N. Abramzon, 2nd Int. Workshop Cold Atmos. Pressure Plasmas, Brugges, Belgium (2005) 69.

[2] K. Becker, A. Koutsospyros, S.M. Yin, C. Christodoulatos, N. Abramzon, J.C. Joaquin, G. Brelles-Mariño, Plasma Phys. Control. Fusion **47** (2005) B513.

[3] N. Abramzon, J.C. Joaquin, J.D. Bray, G. Brelles-Mariño, IEEE Trans. Plasma Sci. **34** (2006) 1304.

[4] K.G. Vandervoort, N. Abramzon, G. Brelles-Mariño, IEEE Trans. Plasma Sci. **36** (2008) 1296.

[5] J.C. Joaquin, J.D. Bray, C. Kwan, N. Abramzon, K. Vandervoort, G. Brelles-Mariño, Microbiology **175** (2009) 724.

[6] G. Brelles-Mariño: Biological and environmental applications of gas discharge plasmas, eds. G. Brelles-Mariño, Nova Science Publisher (2010).

[7] A. Zelaya, G. Stough, N. Rad, K. Vandervoort, G. Brelles-Mariño, IEEE Trans. Plasma Sci. **38** (2010) 3398.