Study of the behavior of the Slime Mold *Physarum polycephalum*, so called Blob, under Cold Atmospheric Pressure Plasma Jet treatment

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Abstract: The use of animal models for research in biology and medicine may face critical issues in the coming years. In this context, we are interested in the slime mold, a non- neural very large unicellular organism, which presents interesting characteristics as a new model system. In this work, we study the behaviour of the slime mold *Physarum Polycephalum* exposed to cold atmospheric pressure plasma jet treatments in two of its living stages, sclerotium and plasmodium, focusing on potential toxicity. Results shows that this organism can undergo severe treatment conditions usually only supported by larger and more complex organisms, hence opening new routes for experiments in plasma medicine and biology.

Keywords: Plasma jet treatment, Slime mold, Physarum polycephalum, behavior, plasmodium, sclerotia

1. Introduction

The last two decades have seen an impressive increase in work dedicated to the applications of the non-equilibrium plasmas at atmospheric pressure, especially in fields related to biology and medicine, leading to numerous works including *in vivo* experiments. Use of animal models in experiments has raised serious concerns in translational research in medicine, especially in Europe. In this context, efforts are being made to find alternatives to the traditional *in vivo* phase in various studies. Currently, organoid and 3D cellular structures *in vitro* are increasingly being used in translational research, however they only mimic part of the organism under study, not the entire organism. Here, we propose to use the acellular slime mold *Physarum polycephalum* (*Pp*), also commonly named blob, as a new model system. *Pp* is a large polynucleated unicellular organism (up to several hundred square centimeters) [1] that responds to its environment and exhibits complex behaviors [2]. Beside plasma medicine, the use of slime molds as *in vivo* models will be of interest for any biological plasma applications, including plasma agriculture, plasma decontamination and plasma cosmetics. They will be also of high interest for the study of plasma assisted drug or particle delivery at topical or cellular level [3].

This work aims to measure the impact of non-thermal plasma at atmospheric pressure and of the associated pulsed electric field CNTs exposure on P. polycephalum performances. The experiments on *Pp* LU352, MALU and DW were carried out with a µs pulsed helium Plasma Gun [4] working at frequencies ranging up to 20 kHz and a reactor high voltage ranging up to 20 kV. We will present results on both the viability and the growth of *Pp* LU352, MALU and DW, in two of their living stages, as sclerotium and plasmodium, in interaction with the plasma. Regarding plasmodia, we focused on the potential of *Pp* to grow again after the treatment and we estimate the how long can *Pp* sustain the treatment without showing any irreversible damage. As for sclerotia, we focused on the capacity of *Pp* to be revived after proper humidification process following the treatment.

2. Materials and methods

2.1 Slime molds *Physarum Polycephalum*

The living organisms under study are slime molds *Physarum polycephalum* of various strains: LU352, MALU and DW. *Physarum polycephalum* is an acellular slime mold that inhabits shady, temperate and damp areas, such as the forest litter. Its haplodiplophasic life cycle is composed of several stages, including spores, plasmodia, sclerotia and fruiting bodies [5, 6]. In our experiments, we used both plasmodium and sclerotium stages. The plasmodium is the vegetative, active, growing and feeding stage. The plasmodium is a vast multinucleate amoeboid cell that crawls over damp wood, leaves or soil, ingesting bacteria, moulds and fungi. When exploring its environment, the plasmodium extends tubular structures called pseudopods. Under adverse conditions, such as food shortage, desiccation or low temperatures, the plasmodium converts into a dormant stage called the sclerotium. This dehydrated and hardened structure may revert to a plasmodium when favourable environmental conditions return. Sclerotia of *Pp* were used to obtain cultures of plasmodia (from CRCA-CBI, Toulouse, France). The plasmodia were cultured in Petri dishes on a 2% agar gel containing 10% of blended oat flakes (Quaker Oats Company), at room temperature in the dark.

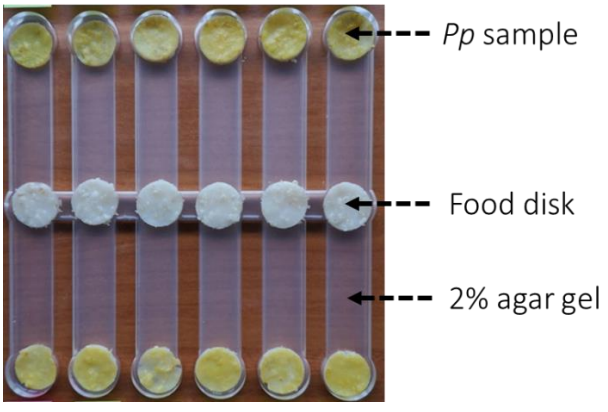

Fig. 1. Parallel blobodrome used in the experiments

Slime molds were taken from the culture using a template (diameter: 20 mm) and introduced in experimental arenas specially developed for these experiments. These arenas, called blobodrome, were machined from Lexan® plates. They consist of multi 18 mm wide channels linked to two wells 21 mm in diameter, all of them being 8 mm deep. They were arranged either in parallel (12 channels) (see figure 1) or in a star shape (8 channels) with a common central well filled with 2% agar gel. The Pp template (also called blob disk below) were deposited in the external wells while the food (2% agar gel containing 10% of blended oat flakes) was placed in the central ones. The growth of each treated blob disk was compared to a control one without treatment. As for the dry sclerotium stage, sclerotia were directly plasma treated, then placed in the different wells after being humidified inducing the transition to the plasmodium stage. Non-treated humidified sclerotia served as controls.

2.2 Plasma treatments

The plasma jet used in this work was a Plasma Gun (PG) [4]. The PG is a coaxial dielectric barrier discharge reactor with a quartz capillary, in this work flushed with helium and powered by µs duration voltage pulses. We used a 10 cm long dielectric quartz capillary of 4 mm/6 mm inner and outer diameters, respectively. In the PG configuration, a hollow high voltage electrode (0.8 mm inner diameter) is inserted inside the capillary, through which helium is injected. A ground ring electrode is set on the outer surface of the capillary overlapping the inner electrode. In this work, the helium flow rate was set at 1.5 L/mn. The plasma treatment was performed using 2 µs (fwhm) high voltage pulses of 6 kV at a repetition rate of 2 kHz. The blob disk samples were placed 12 mm under the capillary outlet. The *Pp* underwent three types of treatment (as shown in figure 2): 1) direct plasma exposure; 2) Only helium gas exposure; 3) only electric field exposure (HV at 14 kV in that case). Treatment duration varied from 30 seconds to 15 mn (longer times will be experienced in the future).

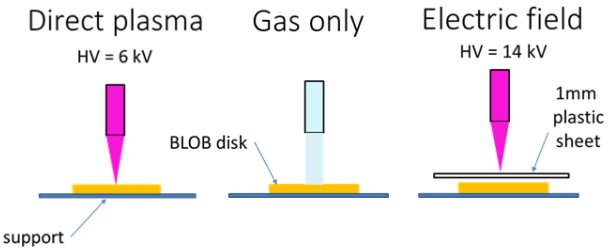

Fig. 2. Configuration of the three types of treatments of the *Pp* samples

3. Results

As shown in Figure 3, for LU352, plasmodia could easily survive to long plasma treatment without losing the capacity to grow, or move toward the food. For treatment lasting less than 1.5 minute, they almost behave as non-treated ones. For treatment time from 90 s to ~ 6' (which corresponds to 720000 discharge pulses), they were slowed for a period of time varying from 10 to 30 hours, respectively, then they behaved normally, being able to generate large plasmodia serving as source for new *Pp* disks. They could not survive for longer treatment times. The experiments showed that long exposition time to helium flow only (true also for argon) was deleterious and was the main reason for the observed behaviour. Experiments on MALU and DW brought the same observations as the LU352 ones. The blobs disks exposed to the field only (1.4 kV/cm at the sample level) for less than 15'were not perturbed and behave like controls. An amazing result was that slerotia of the three *Pp* types could easily undergo 15' treatment of direct plasma. After proper humidification, they could be turned into new plasmodia without any problem. All of these results, and other non-mentioned here but that will be presented, reveale the ranges of plasmas parameters and treatment time that will be of interest for further experiments on blobs without endanger their survival.

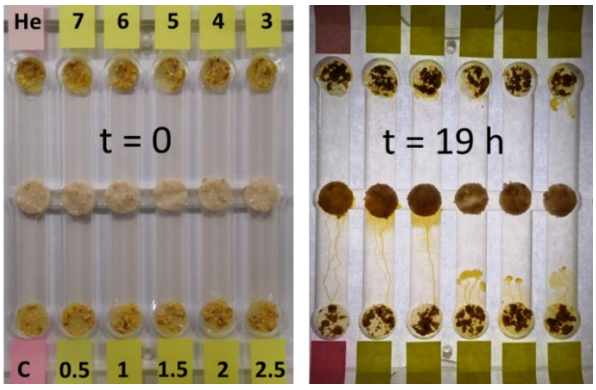

Fig. 3. Treatment of *Pp* LU352 plasmodia : growth at 19 hours compared to the initial situation just after treatment. Numbers indicate the treatment time in minutes; C for Control; He for helium gas only during 15'

4. Conclusion

The experiments presented in this work showed that slime molds *Physarum polycephalum* are extremely resistant to cold atmospheric pressure plasmas treatment in both their plasmodium and sclerotium stages. These results demonstrate that slime molds constitute an interesting model to plasma medicine and to biology in general.

5. References

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