NON THERMAL ATMOSPHERIC PLASMA JETS : A NEW WAY FOR CANCER TREATMENT?

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ABSTRACT

Number of publications report on non thermal plasma (NTP) potentialities for biomedical applications. Cancer treatment appears as one of the most promising. There are still many steps to go on before clinical applications but the route is now clearly open. We will go through what have been already demonstrated concerning *in vitro* and *in vivo* experiments, what are the challenges in front us, what problems are directly linked with discharges and finishing trying to draw what are the main perspectives in the domain.

1. INTRODUCTION

Research on biological applications of NTP is an extremely fast growing domain at the moment, demonstrating new potentialities almost every day in sterilization and decontamination, biomaterials developments and engineering, agricultural applications and diseases treatments. Among those last ones, studies related to cancer treatments [1]are taking an increasing place and now are the main goal of a rapidly increasing number of teams all around the word. Mainly, studies performed at atmospheric pressures are ran using Dielectric Barrier Discharges (DBD) or Plasma Jets (PJ) directly linked to the a DBD reactor or allowing formation of plasma at long distances through dielectric capillaries such as the Plasma Gun (PG).

The generated atmospheric pressure plasma, directly in air (DBDs) or resulting from rare gas plasma transfers to surrounding air, are intense source of production of Oxygen and Nitrogen Reactive Species, ROS and RNS respectively, that are know to play an important role on the biological matter. NTP effects on cells and micro-organisms are now well known, but the comprehension of the mechanisms involved in the observed effect in disease treatments is far from being reached, together with potential combined effects of microenvironments of cells or tissues due to potential local changes of pH or influence of the local electric field. So beside the already obtained results, both *in vitro* and *in vivo*, a lot more work as to be done to develop, to characterize, and to optimize dedicated plasmas devices and to study their effect on living organisms before or to accompany clinical tests or real clinical treatments and procedures.

In this presentation, we will first rapidly present the different results obtained on the antitumor effect in vitro and in vivo. Then we will concentrate on the plasmas devices themselves and their characterization that must be carefully considered if we want to understand or optimize what is observed. In a last part, we will try to present new developments of interest for the domain and new perspectives that can envisaged.

2. PREVIOUS RESULTS ON ANTITUMOR EFFECT *IN VITRO* AND *IN VIVO*

The antitumor effect of NTP is now well established *in vitro* (for example see ref. [1], [2] Fig.1). Large number of tumorigenic cell lines have exposed to various type of NTP. In almost all cases, it has been shown a strong effect on cell viability, NTP inducing necrosis or apoptotic (programmed cell death) behaviour on rather

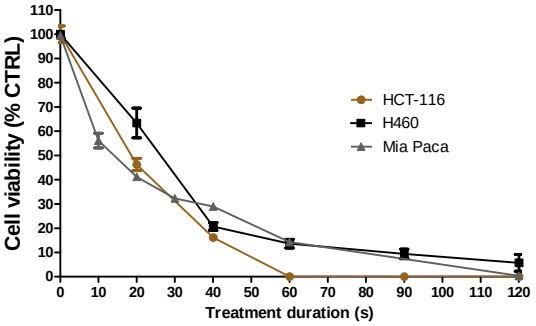

Fig. 1. Effect of PG treatment on tumor cells in vitro (helium at 400 sccm): HCT 116 (colon), H460 (lungs), Mia Paca (pancreas)

short times (minutes to few seconds) depending not only on the used plasma device (afterglow, DBD, plasma jets respectively), but also on the used type of buffer solution in which cells are maintained. To date (see [1], [2], [3]), we can mention (non exhaustive list) that an antitumor effect has been proven *in vitro* on carcinogenic cell lines related to cancer of skin (melanoma), brain (glioblastoma), colon, liver, lungs, breast, cervix, bladder, or seen in oral and ovarian carcinoma or in leukaemia

NTP antitumor effect has been shown *in vivo* on a smaller variety of tumors. This is not due to a lesser effect, but mainly cause by the complexity to run the experiments on dedicated animal models (most of the experiments have been ran using Balbe C nude or Black C6 mice) with enough individuals per groups (control group, modes of treatment groups). We can mention that very promising results have been obtained on melanoma [4], [5] glioblastoma [6], colorectal carcinoma [2], blader carcinoma, ovarian carcinoma [7] and pancreatic carcinoma [8]directly or through plasma activated medium [7]. The processes involved in the chain leading to reduction of tumor growth are far from being understood, but we can now think that at least ROS are playing an important role [2]. With NTP, we observed a similar result to radiotherapy, with the formation of DNA damages in treated cells, leading to cell cycle arrest, followed by caspase activation, and finally to cell death with both early and late apoptosis.
Taken together, these results suggest that NTP could be a new strategy against cancer cells, more especially when used in combination with antitumor drugs. Fig. 2 is a striking example of what can be obtained when combining atmospheric NTP, in this case delivered by a Plasma Gun, with chemiotherapy. In that experiment, ran in the frame of the PLASMED project, pancreatic tumors have been treated *in vivo* in various conditions [8]. Experiments were carried out using four mouse groups: one control group, one group treated only with Gemcitabine (200mg/kg), one group treated only using the Plasma Gun, and one group treated using a combination of Gemcitabine (200mg/kg) and Plasma Gun. One can see on Figure 2, that the reduction of tumor volume at Day 36, compare to control group, is much more important in the case of the group treated with plasma gun (68 %) than in the case of the group treated with chemotherapy (25 %). The striking results of these series of experiments is that, not only, the plasma treatment appeared more efficient than Gemcitabine alone, but that the combination of both led to a most effective tumor growth inhibition (mass reduction of 87 %) supporting the possible interest to use NTP in combination with a chemotherapeutical agent. This indicates that probably, the modifications of the microenvironment of the tumor by the plasma is of primary importance suggesting that more attention must be paid to the local induced modifications, as we will see in the last part, and so that great care must be taken in producing, and applying ROS and RNS, also in the transient electric field environment, on the target. This is directly link to the plasma production in living target environment itself.

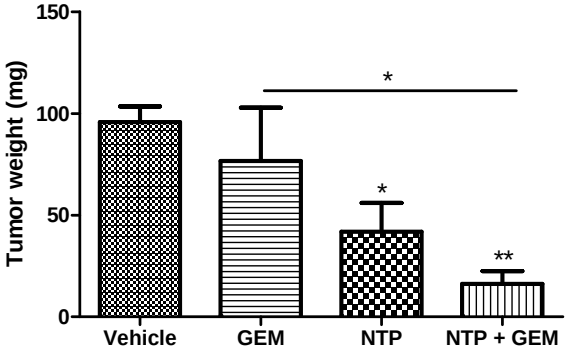

Fig. 2. Pancreatic Carcinoma Effect on tumor weight the day of euthanasia (D36); four mouse groups: Vehicle, control; GEM, treated with gemcitabine; NTP, treated with Plasma Gun; NTP+GEM, treated with both plasma and gemcitabine

3. DISCHARGES AND PLASMA DEVICES

As just mentioned, it appears that a good control of RS species production and deposition is of primary importance in the tumor treatment. This is also true for any kind of living targets which in turn have also an effect on the atmospheric plasma development [9]. For example, in the case of Plasma Jets, both the structuration of the rare gas flow depending on the discharge parameters and the nature of the living target must be taken into account. It is clear that wrong estimations of RS populations delivered can be derived if they are obtained from measurements done in jets expanding freely in air or without the proper target in front of the PJ. Examples can be given concerning shape of the flow and mixing of the rare gas flow with the surrounding air. A good example is given in Fig.3 (Schlieren

imaging) where one can see the huge difference of the flow shape of helium (0.5 slm) exiting from a PG with or without plasma on.

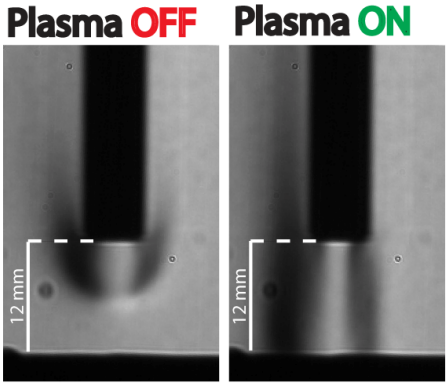

Fig. 3 Helium flow structuration by the plasma produced by a PG. He (0.5 slm); Grounded target at 12 mm from the capillary outlet.

In Fig. 3, it is obvious that comparison, as it is often done in the literature, between plasma OFF and plasma ON must be done carefully because in the first case, the gas flow doesn't reach the target, while it does in the second one. Other striking difference is the behaviour of the rare gas flow without target, or with floating or grounded target [10]. From Fig. 4, it is clear that diagnostic of the plasma expending freely in air won't give any idea of RS production in presence of a target, and even more, that this production will considerably vary with the nature of the target. This is clearly shown (Fig 5) when combining ICCD imaging and LIF diagnostic in the case of plasma produced by a PG impigning or not on a target of given conductivity, in that case saline water [11].

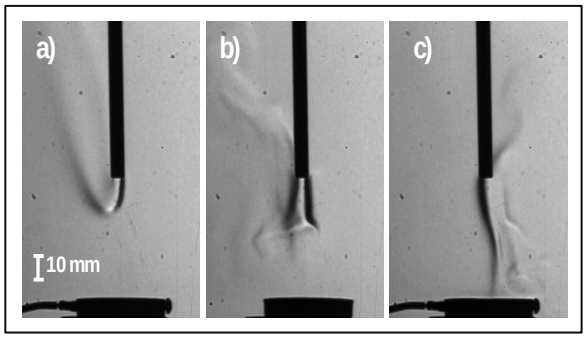

Fig. 4 Gas flow structuration by plasma depends on target conductivity: a) without plasma; b) plasma with target at floating potential c) plasma with grounded target; He 1slm, 14kV, 2 kHz [10]

Those results emphasize the fact that the RS densities are strongly dependent upon the discharges parameters and show how great care should be taken in establishment of set ups similar to biomedical treatment conditions for plasma jet *in-situ* diagnostics. Indeed this type of studies must consider not only the jet input parameters as voltage amplitude and gas flow rate, but also the target characteristics, i.e. both its physical (electrical conductivity) and chemical nature.

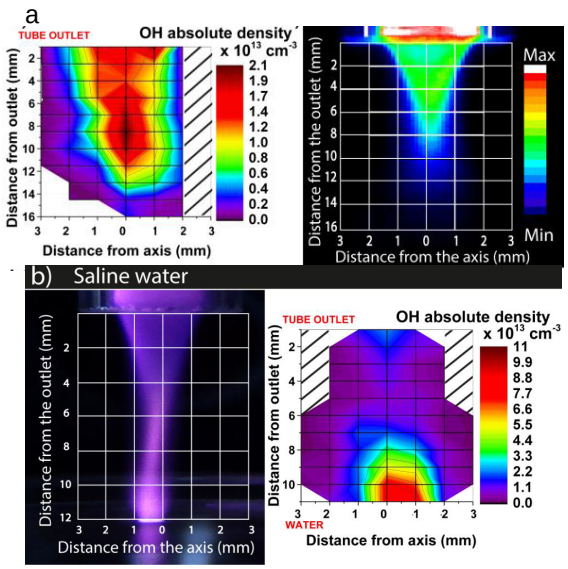

Fig 5 LIF measurements of the OH density in a plasma jet produced by a PG: a) plasma expending freely in air; b) Plasma in presence of a liquid saline water target. In both cases, the PG parameters are: 2 kHz,14 kV, He 1 slm.[11]

4. PERSPECTIVES

As mentioned previously, the antitumor effect of NTP has been clearly shown *in vivo* on murine models with various cancer types. Although the mechanism is far from being fully understood, the therapeutic effect is admitted. To date, the literature reports mainly induction of apoptosis, effect which is not restricted to cancer cells and probably mask other aspects of the NTP for cancer therapy. Now research has to focus on "soft" applications allowing highlighting interesting effects as recently shown [12] on blood vessel parameters: blood flow and tissue oxygenation. For example, in our recent work various plasma conditions were established to allow a daily treatment without skin burn or damage. PG helium NTP used in this work [13] not only leads to the production of reactive species, but also generates transient electric fields. Immuno-competent balb/c mice were used to study the proliferation of orthotopically implanted 4T1 breast carcinoma (Mice were double grafted. One tumor was used to apply a specific treatment condition whereas the second tumor of the same mouse was used as control

tumor (untreated)). Preliminary data show a regulation of tumor proliferation regardless the variations among the treatment conditions applied (connected or not to the ground, treated through H2O impregnated compress or directly, 2 kHz *vs* 200 Hz). This observation is in favor of a triggering effect, evidenced for the first time, of the plasma fraction which similarly affects the tumor proliferation (treated group in Fig.6) as compared to untreated tumor (contra-laterally grafted on each mice). This comparable reduction of tumor proliferation obtained whatever the plasma soft treatment conditions, with a significant reduction of standard deviations in all cases, clearly indicates a tumor growth regulation. Taking into consideration the recent vessel normalization based-cancer treatment, the NTP effect should be further investigated in view of blood vessels structure and function (blood flow) as well as tumor hypoxia compensation to confirm a possible NTP-based adjuvant approach for cancer treatments. These results suggest a new way to consider the plasma and its therapeutic delivery in NTP-based tumor therapy, also combined with endoscopic treatments [14].

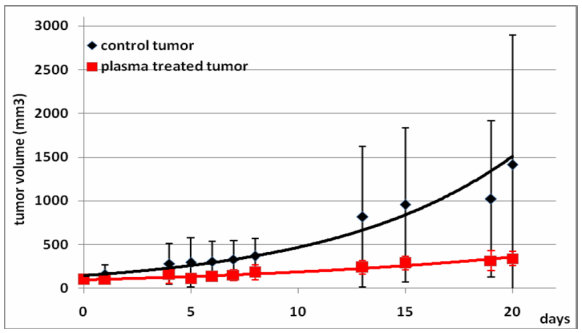

Fig 6 In vivo study of 4T1 breast carcinoma proliferation with distinct treatment conditions (see text)

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