

# Cell death Mechanism on human colorectal cancer after PAM (Plasma Activated Medium) treatment

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Cell death mechanism was investigated on HCT116 MCTS (Multi-Cellular Tumor Spheroids) with a luminescent analysis of the cell viability by measuring the Adenosine triphosphate (ATP) rate in cells. A fluorescence analysis was also conducted to investigate DNA damage, cell permeabilization and caspase detection as a mean to indicate apoptosis. After a several hours in Plasma Activated Medium (PAM) cells begin to detach from the MCTS as the level of ATP decreases. This loss in ATP can be attributed to the decrease in living cells. At the same time an increase in caspase intensity is occurring as well as cell permeabilization which are characteristic of apoptosis.

## 1. Introduction

Cold atmospheric pressure plasmas have received a growing interest in the past few years in the biomedical field and more precisely cancer treatment [1]. Recently, some studies used plasma activated medium (PAM) and have demonstrated an interesting effect on cancer cells like a decrease of cell proliferation [2], DNA damage [1] and apoptosis [3]. The main advantage of PAM is its possibility to be prepared in advance and then stored at the right temperature in order to be used later since the aqueous plasma byproducts can remain active up to several days [1].

The present work investigates the effect of PAM on HTC116 MultiCellular Tumor Spheroid and cell death mechanisms. MCTS is a model that mimics the 3D organization and the regionalization of a micro-tumor region. The medium was activated with a plasma jet based on a dielectric barrier discharge configuration excited by high voltage square pulse with a Helium gas flow fixed at 3L/min.

## 2. Results

The present work investigates cell death mechanism occurring in human colorectal cancer tumor spheroids after PAM treatment. Fig 1 shows light images of MCTS HCT116 spheroids after PAM treatment and a cell detachment is observed between 4-6H after treatment.

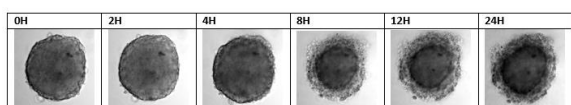


Fig 1. Light Images of MCTS HCT116 after PAM treatment.

In order to understand this cell detachment, early cell death mechanism was investigated. ATP levels were quantified and a rapid decrease as soon as in the first 30 min post-treatment was observed (Fig 2).

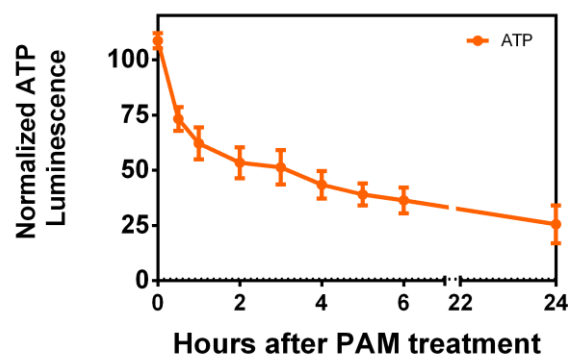


Fig 2. Normalized ATP luminescence evolution after transfer of MCTS into PAM (Cell viability evaluated by ATP luminescence).

DNA damages were also investigated and have previously been demonstrated to be linked to the presence of hydrogen peroxide inducing this cell detachment. [1]

Fluorescent analysis of cell permeabilization and apoptosis were conducted underlying apoptosis as the main cell death mechanism.

## 3. References

- [1] F. Judée *et al.* *Sci Rep*, **22**, 21421 (2016)
- [2] N. Hattori *et al.* *International Journal of Oncology*, **47**, 1655-1662 (2015).
- [3] K. Torii *et al.*, *Gastric Cancer*, **18**, 635-643 (2014)