COLD PLASMA DEPOSITION AS A NOVEL TECHNOLOGY FOR TARGETED CANCER DRUG DELIVERY

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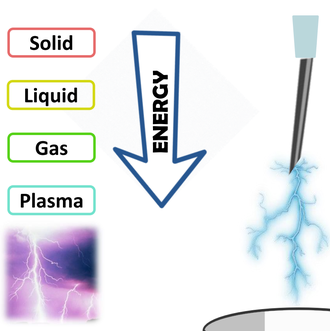
Introduction

Plasma is a reactive mix of:



Reactive species

UV Radiation

Heat Radiation

Ions / Electrons

Electromagnetic Fields

Visible Light

Could be applied to:

Plasma Activated Liquids (PAL)

Potential to become a controllable targeted approach

Glioblastoma multiforme (GBM) is the most common, malignant and aggressive brain cancer[1].

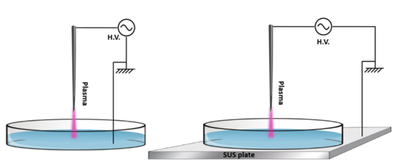
Topotecan (TPT) is an antineoplastic agent with major **cytotoxic effects during S-phase of the cell cycle** (inhibiting DNA topoisomerases I)[2].

Research Aim: Development of a combined therapeutic approach using TPT and Plasma based technologies for Glioblastoma.

Methodology

Plasma Activated Liquids (Part A)

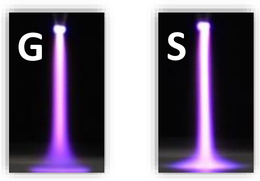
Systemic treatment



(a) Glow discharge (b) Spark discharge

RSS Device

AC air discharge system
6–10kV, 25kHz, 5mm gap



Plasma treatment
10ml of water treated for 15 minutes with Glow and 5 minutes with Spark

Targeted deposition of anti-cancer agents onto cancerous tissue

Oncology drugs (Part B1)

Evaluate the effect of oncology drugs alone

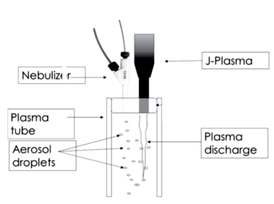
Combinatorial approach (Part B2)

Cell line
U-251mg (human glioblastoma) cultured in DMEM/F12 + 10% FBS, supplemented with 20 or 5%(v/v) PAW

Long term survival: Clonogenic assay

Plasma Deposition (Part C)

In situ treatment



J-Plasma Device

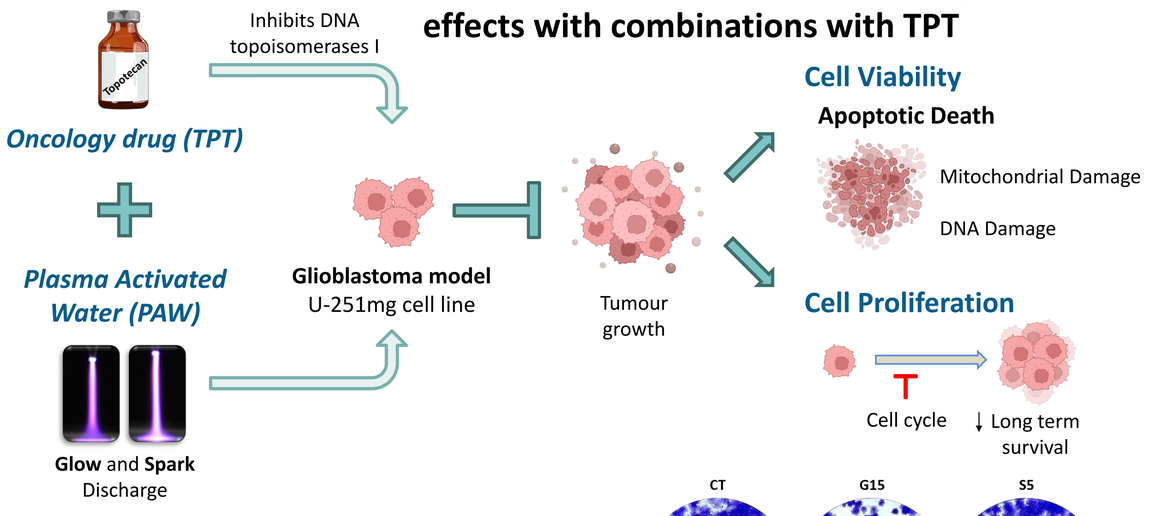
Selectivity of treatment (Part D)

Assays
Metabolic Activity: Resazurin assay
Cytotoxicity: Crystal Violet assay

Flow Cytometry
Cell Death: Annexin V / 7AAD
Cell Cycle/Proliferation: PI/EdU

Results

Parts A and B **Establish parameters for PALs, evaluate the toxicity profiles and determine the potential synergistic/additive effects with combinations with TPT**



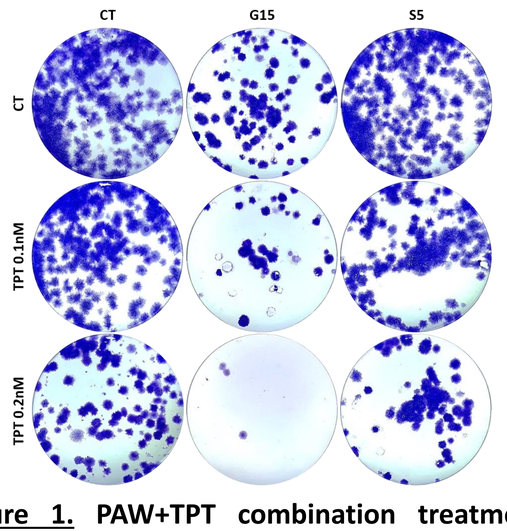


Figure 1. PAW+TPT combination treatment decreases long term survival. U-251mg cells were treated for 72h either with PAW, TPT or PAW+TPT. Colony formation was evaluated 14 days after the end of treatment.

Combined treatments between PAW+TPT :

- Reduce metabolic activity and cell mass
- Increase apoptotic cell death.
- Contain an increased number of cells in S phase and G2/M of the cell cycle

Indicate a cytotoxic effect in short term and an anti-proliferative effect in long term in U-251mg glioblastoma cells.

Results

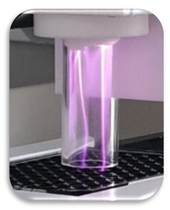
Parts C and D **Establish optimal deposition parameters and concentrations for TPT deposition onto cells; TPT analysis for changes to structure and/or function using *in vitro* cell assays**

New therapeutic approaches → **Plasma Deposition**

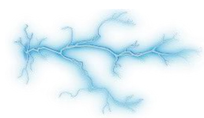
*Repurposing current drugs **(TPT)*** → **Plasma Deposition of TPT**

TPT flow at 10 µl/min
Helium flow of 2 slm

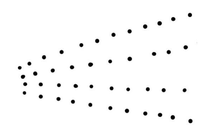
Redline G2000 High Voltage (HV)
Output of 90V
Constant frequency of 19.4 kHz
45% duty cycle
Helium flow of 6 slm



Plasma activated



TPT wet coating



TPT Plasma deposition

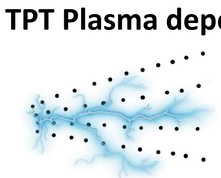


Figure 2. TPT Plasma Deposition treatments presented a more consistent cytotoxic effect in U-251mg cell line than the wet coating. These results were obtained after 72h treatment with media eluted from the treated plates, by Resazurin and Crystal Violet assays. Statistical significance is represented as: ****P<0.0001.

Future work:

- ***In vitro* evaluation of the effect of TPT plasma deposited**

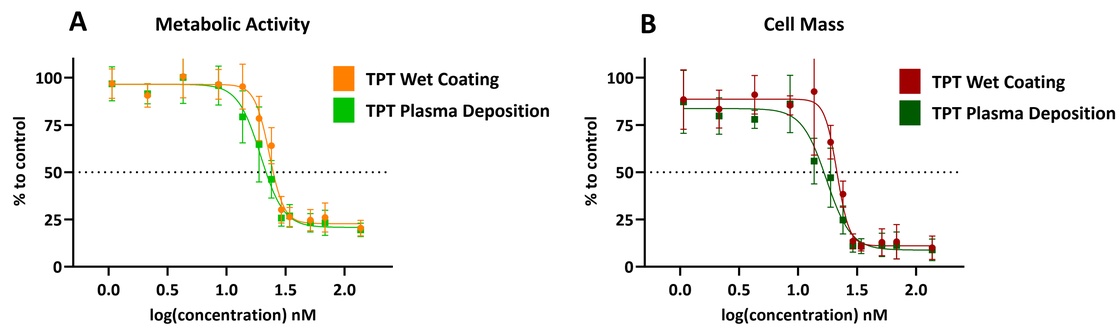


Figure 3. Dose-response effect of TPT Wet Coating and TPT Plasma Deposition in U-251mg cell line. (A) IC50 curves for metabolic activity; (B) IC50 curves for cell mass.

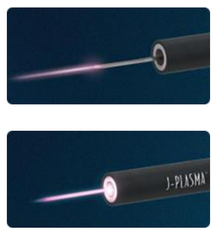
- **Identification of a presence or absence of TPT selectivity on the selected paired tumour/control cell lines**

Possible future applications

J-plasma is already FDA approved for:

- cutting, coagulation and ablation of soft tissue
- open and laparoscopic cases
- tumour removal

Retractable blade



Nebulizer

Margin treatment after tumour resection

References

[1] Bernstock, J.D. *et al*, *Scientific Reports,* 2017;
[2] Ling, Y.H. *et al*, *Cancer Chemother Pharmacol*, 2001;

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