Fluorescent detection and photodynamic therapy of cancer using 5-Ala induced PpIX and a scanning fiber endoscope

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Introduction

- 70,000 new cases of bladder cancer with about 15,000 deaths per year in the US. [1]
- Bladder Cancer is the most expensive cancer for a per-patient cost from diagnosis to death. Costing approximately $96,000 to $187,000 US dollars.[2]
- Recurrence rate of 50-70% [1]
- Photodynamic therapy (PDT) is one of many cancer treatment options being researched. It is a photochemical reaction between a photosensitive molecule in the cell and light. The reaction releases a singlet oxygen molecule that causes cell death through apoptosis or necrosis.
- This research tests PDT with 405nm light and 5-aminolvulinic acid (5-ALA) induced protoporphyrin (PpIX).
- When 5-ALA is applied exogenously to living cells it converts to PpIX preferentially in cancer cells.

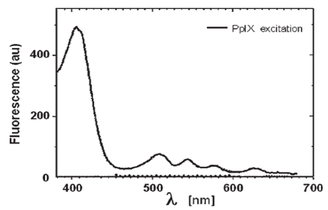

Figure 1.1 PpIX Excitation spectrum [5]

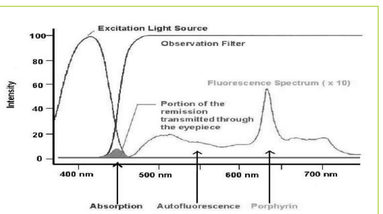

Figure 1.2 PpIX Emission Spectrum[5]

- When PpIX is excited by 405nm (violet) light it will emit at a maximum of 635nm, appearing red.
- For therapy, illumination will be conducted using a Scanning Fiber Endoscope (SFE) created in the Human Photonics Lab (HPL) in at the University of Washington.

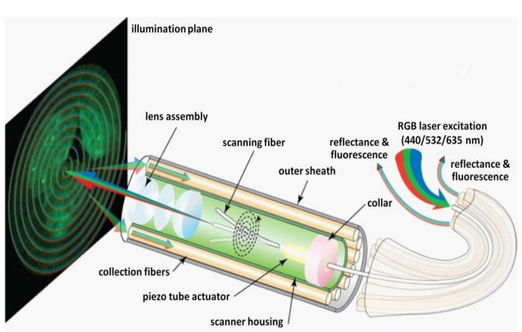

Figure 1.3 Scanning Fiber Endoscope[3]

Results of Fixed ALA Concentration

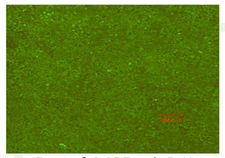

Figure 3.1 PDT with 5.4J

Control (No 5-ALA)
300s @ 0.018W
Energy: 5.4J
Area of Death: None

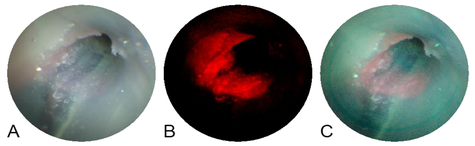

Figure 3.2 (A) Standard RGB image (B) Fluorescence emission image (C) Mixed Green and blue reflectance and red fluorescence. [3]

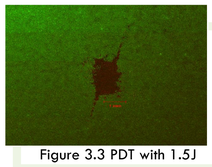

Figure 3.3 PDT with 1.5J

300s @ 0.005W
Energy: 1.5J
Area of Death: 263.36 cm^2

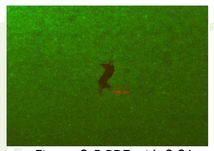

Figure 3.5 PDT with 0.9J

60s @ 0.015W
Energy: 0.9J
Area of Death: 80.78cm^2

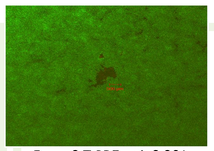

Figure3.7 PDT with 3.38J

180s @ 0.0188W
Energy: 3.38J
Area of Death: 74.65 cm^2

Δ=87.28cm^2 Δ=33.14cm^2 Δ=43.83cm^2

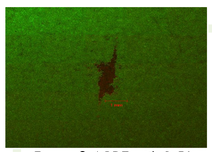

Figure 3.4 PDT with 1.5J

75s @ 0.02W
Energy: 1.5J
Area of Death: 176.08 cm^2

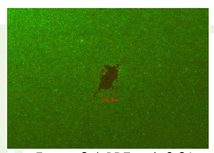

Figure 3.6 PDT with 0.9J

180s @ 0.005W
Energy: 0.9J
Area of Death: 113.92 cm^2

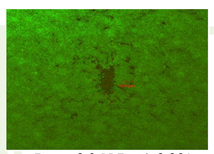

Figure 3.8 PDT with 3.38J

338s @ 0.01W
Energy: 3.38J
Area of Death: 118.48 cm^2

Methods

Cell Culturing:
A549 cells, donated from VisionGate Inc (Phoenix, AZ), were used to run experiments. The cells were initially grown in cell culture flasks using a DMEM base growth medium with 10% FBS and 1% Penicillin and Streptomycin. When experiments were to be performed the cells were removed from the flasks and seeded onto glass chamber slides. Cells were allowed to grow until a confluent monolayer was achieved.

5-ALA administration:
To run experiments, a 5mM solution of 5-ALA, obtained from Organix UK of the University of Essex, in Serum Free DMEM was used. The cells were incubated in the solution for 4 hours. At the end of the 4 hours the 5-ALA solution was removed and the cells were rinsed twice with PBS before additional PBS was used to fill the chamber slide for PDT experiments.

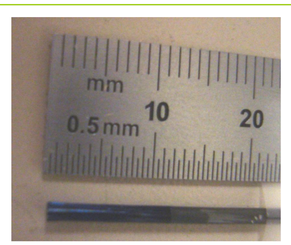

Figure 2.1 Scanning Fiber Endoscope

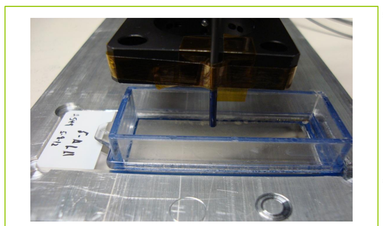

Figure 2.2 Experiment Setup

Photodynamic Therapy:
For PDT experiments a test setup was built to ensure repeatable experiment conditions. The confluent monolayer of cells in PBS, were then illuminated by scanning 405nm laser light for a variety of time durations and intensities. These time durations and intensities were chosen to compare equal amounts of energy delivered to the cell culture (J/cm^2) with 405nm light. Time of therapy ranged between 1 and 5 minutes while light intensity ranged from 5 to 20mW.

Analysis of Results:
To view the results of therapy, LIVE/DEAD® solution, from Invitrogen, was used in conjunction with confocal microscopy. Using this approach, live cells could easily be distinguished from dead cells and the area of cell death could be determined.

Conclusion

The results show that PDT of A549 cancer cells using 405nm light and 5-ALA induces PpIX was successful. The SFE was able to direct therapy at specific areas within the device's field of view and avoid causing cell death in unseen/unexposed locations. The results also show that with increased durations of therapy, the area of cell death increased.

Previous research by V. H. Fingar et al [4] shows that an increase in drug dose and a decrease in light dose and vise versa will yield equal results, thereby creating a correlation between light dose and drug dose. In this research, the results show another correlation between light intensity and length of exposure. It was observed that an increase in time of expose and a decrease in optical power such that total power is kept constant will yield a larger area of cell death.

Future Direction

Future research will be performed with a better calibrated SFE to allow for a more consistent spatial and temporal light delivery. This should allow for a more precise method for quantifying cell death based on total light delivery.
Co-cultures, with healthy and cancerous cells, will also be studied to determine the difference in effects of PDT between cell lines.

Acknowledgements

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References

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