A. James Clark School of Engineering
Fischell Department of Bioengineering

Children's National

Photodynamic Therapy as an Alternative to Tonsillectomy Procedures

Justin Sylvers, Arley Wolfand, N. Zachary Rausch, Natalia Ochman, and Jason Chiang

Faculty Advisor: Dr. Huang Chiao Huang, Fischell Department of Bioengineering, University of Maryland

Clinical Mentors: Dr. Kevin Cleary, Dr. Avinash Eranki, and Dr. Rahul Shah, Children's National Medical Center

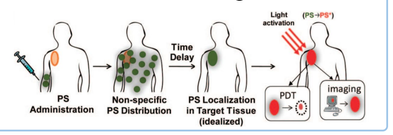
Motivation

Tonsillectomies, a procedure performed to remove tonsils due to infection and sleep obstruction, are one of the United States' leading procedures in children. As of 2011 there were **530,000 performed each year.** Current tonsillectomy procedures, like cold-knife, electrocautery, and microdebrider techniques, have **high pain, risk of hemorrhage, and infection** associated with them.

Background

Steps of photodynamic therapy (PDT):

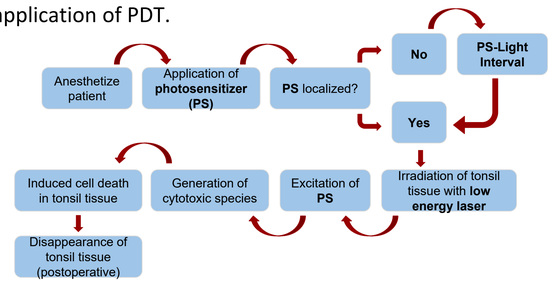
1. Inject/apply a **photosensitizing agent (PS)**
2. Allow for distribution to the target tissue
3. Use a **low-powered laser** to activate the drug
4. Generate cytotoxic species and **induce cell death** through mechanisms that vary with PS



Objectives

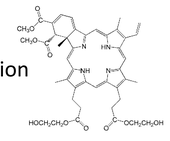
Experimentally validate the use of photodynamic therapy (PDT) as an alternative approach to tonsillectomy procedures:

1. Evaluate the ability of the photosensitizer to **penetrate** excised tonsil tissue with varying concentrations and time considerations.
2. Evaluate the amount of **cell death** in excised tonsil tissue caused by application of PDT.



Materials

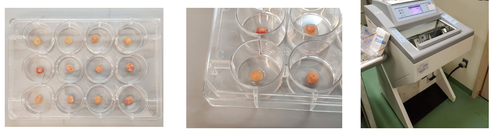
- Excised tonsil tissue acquired from Children's National Medical Center with IRB approval.
- Liposomal Benzoporphyrin Derivative (L-BPD) in phosphate-buffered saline (PBS) ranging in concentration from 0.5 - 70 µM.
- Precision LED Spotlight - Standard Range, 680 nm (Mightex).



Methods

Drug Penetration Studies

- Cut excised tonsil tissue into equal cylindrical portions using a biopsy punch.
- Applied L-BPD solution and incubated.
- Cryosectioned samples and imaged with fluorescence microscopy (**Ex/Em: 435/690 nm**).



Cell Death Studies

Four groups:

(-)BPD (-)Laser, **(+)BPD (-)Laser**, **(-)BPD (+)Laser**, **(+)BPD (+)Laser**

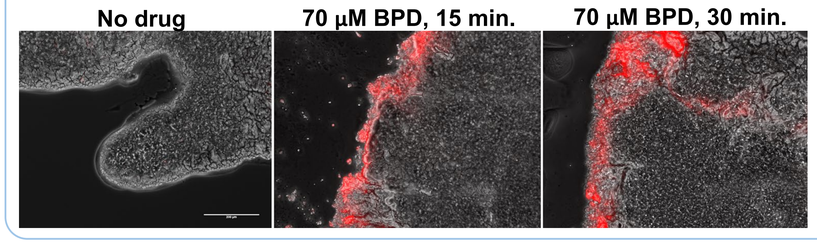
- Prepared cylindrical tonsil portions and applied drug as above using metrics with best penetration (**70 µM BPD solution**, **30 min. incubation**)
- Irradiated with 690 nm laser for 15 minutes at a height of 10cm to a fluence of **~75 J/cm²**
- Cryosectioned samples, applied H&E stain, and imaged with BF microscopy.

Results

Drug Penetration Results

Merged phase contrast and fluorescence images of sectioned tonsil tissue are shown below.

- 30 minute incubation resulted in greater penetration (distance from edge) than 15 minute incubation on average (155 ± 112 µm and 292 ± 223µm). Both were well below the target depth of germinal centers, 1-2 mm.
- As indicated by large standard deviations, the depth of penetration fluctuated significantly. This is attributed to uneven application of BPD solution, with greater penetration observed in areas closer to the location of application.



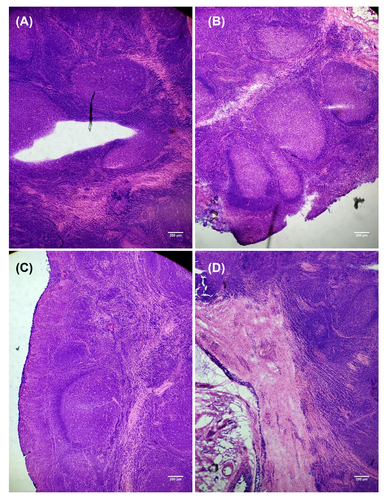
Cell Death Results

Representative H&E images to the right.

A: **(-)BPD (-)Laser**
B: **(+)BPD (-)Laser**
C: **(-)BPD (+)Laser**
D: **(+)BPD (+)Laser**

Necrotic pattern is characterized by light pink staining with sparse nuclei - this is evident in all groups due to inherent death of excised tissue.

Qualitatively, most widespread necrotic area observed in **(+)BPD(+)Laser**, suggesting effective induction of cell death with full treatment.



Conclusions & Future Work

Through our capstone project we have illustrated a possible application for PDT as an alternative to tonsillectomies that has not previously been explored. Our preliminary research suggests that L-BPD is capable of penetrating excised tonsil tissue and, in combination with irradiation, induces cell death. Further work needs to be done to validate the procedure before introducing it to the clinical setting:

1. More precise quantification of cell death to establish efficacy of PDT for removal of tonsil tissue
1. Animal studies to fully characterize the ability of the procedure to remove tonsil tissue and possibly provide insight regarding pain and safety

Ethical Considerations

Application of this procedure would positively impact physicians, patients, and patient families. Patients and families would benefit from decreased pain, recovery time, and risk of hemorrhage. The decreased recovery time and need for return visits would allow physicians to focus on other cases. However, perception of utilizing lasers in a child's procedure carries some stigma, which would have to be mitigated.

Significant References

1. Volk M, Wang Z, Pankratov M, Perrault D, Ingrams D, Shapshay S. Mucosal intact laser tonsillar ablation. *Arch Otolaryngol Head Neck Surg.* 1996;122(12),1355-1359.
2. Celli JP, Spring BQ, Rizvi I, et al. Imaging and photodynamic therapy: mechanisms, monitoring, and optimization. *Chem Rev.* 2010;110(5),2795-2838.