Ultrasound-Enhanced Ocular Drug Delivery for Treatment of *Acanthamoeba* Keratitis

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Introduction

- Usually, less than 5% of therapeutic agents make it through the cornea due to: corneal epithelium barrier properties, normal tear production, and blinking [1].
- The human eye contains several barriers that normally work to stop toxicants from entering the eye and help maintain normal visual function. However, these very methods that work to protect the eye also make drug delivery difficult [2].

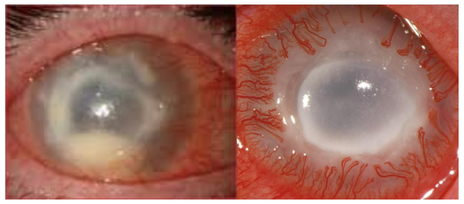

Fig. 1: Appearance of Acanthamoeba Keratitis [3]

- *Acanthamoeba* keratitis (Fig. 1) is a painful and serious infection of the cornea that can lead to permanent vision loss or blindness[3].
- This disease has been on the rise in the United States where it is estimated that 85% of cases occur in contact lens wearers [4].
- We are interested in exploring the possibility of ultrasound-mediated drug delivery as an inexpensive, effective, and minimally invasive treatment.

Current Treatment

- Mainly treated with the biguanides Polyhexamethylene biguanide (PHMB) 0.02% or Chlorhexidine 0.02% in combination with antibiotics, steroids, or biocides.
- Treatment can last anywhere from 3 weeks to a year.
- Frequency of application is every few hours at the beginning of treatment and decreases over time, which leads to low patient compliance.
- An early diagnosis is needed for effective treatment, however most doctors misdiagnose the disease initially, leading to worse outcomes.

Specific Aims

1. Determine the optimal ultrasound parameters for transcorneal ocular drug delivery of PHMB.
2. Histological analysis to determine the ultrasound induced structural damage.

Previous Studies

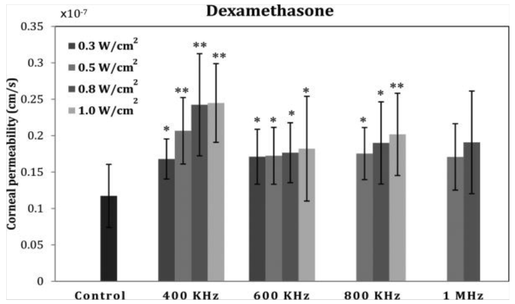

Fig. 2: In virto study results showing that transcorneal delivery of dexamethasone was best achieved at 400 kHz [5]

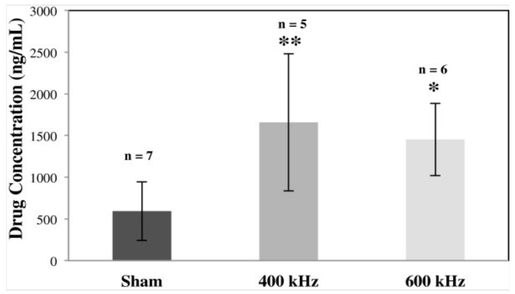

Fig. 3: in vivo study showing that ultrasound application can increase transcorneal delivery of dexamethasone 2.8 times and 2.4 times respectively [5]

Methods

Diffusion Cell Experiments

Dissected rabbit corneas are placed in a diffusion cell setup (Fig. 4) between a donor compartment filled with PHMB and a receiver compartment filled with Dulbecco's phosphate-buffered saline (DPBS). Each cornea was exposed to drug solution for 60 minutes, with the experimental group receiving ultrasound in the beginning of treatment.

Ultrasound Parameters

- 400 kHz or 600 kHz
- 0.5 W/cm^2 or 0.8 W/cm^2
- 1, 3, or 5 minutes of continuous ultrasound
- 5 minutes of pulsed ultrasound at 25% duty cycle

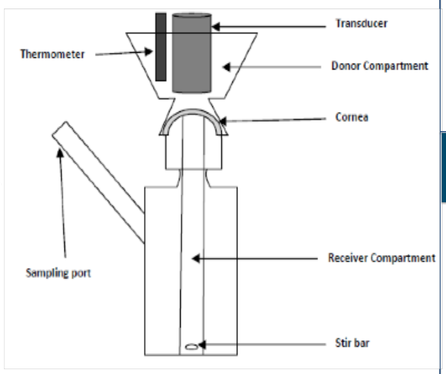

Fig. 4: Experimental Setup for diffusion cell experiments

Spectrophotometry

After each experiment, 2 mL of solution was collected from the receiver compartment of each diffusion cell through its sampling port. Its absorbance was measured using a spectrophotometer.

Histology

Corneas were fixed in formalin after treatment and later sliced into semi-thin sections for histology slides. Images were taken at 10X and 40X magnification with a light microscope. These images were used to visually examine structural changes in the epithelial, stroma, and endothelial layers of the cornea.

Results

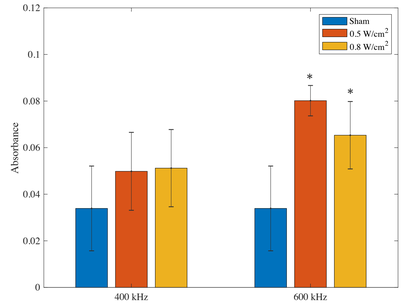

Fig. 5: Five minute treatment group

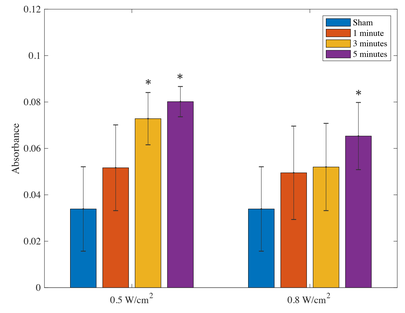

Fig. 6: Decreased time for 600 kHz

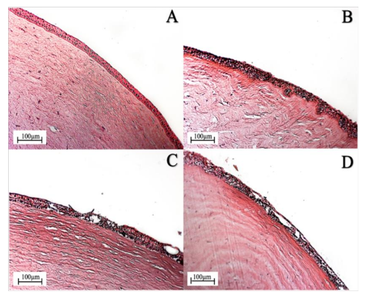

Fig. 7: Histological Analysis. A control cornea (A), a sham-treated cornea (B), a corneal sample exposed to 400 kHz ultrasound (C), and a corneal sample exposed to 600 kHz ultrasound (D).

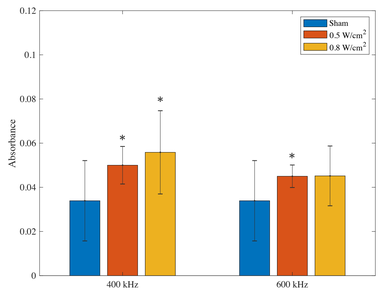

Fig. 8: Pulsed Ultrasound

Future Studies

- Testing more ultrasound parameters
  - 0.3 W/cm^2 - 1 W/cm^2
  - 400 kHz – 1MHz
- Modeling studies to determine the thermal effects of ultrasound exposure in ocular and surrounding tissues at the experimental parameters.
- Confirm experimental results using an antifungal ocular drug.

References

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