Ultrasound-enhanced Delivery of Antibiotics and Anti-inflammatory Drugs into the Eye

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Background

• Delivery of drugs at therapeutic levels is frequently a problem in the treatment of various ocular diseases.

• Topical administration of drugs to the cornea is a preferred route for delivery of ocular drugs.

• Achieving 2-3 times increase in the amount of delivered drugs is considered clinically significant.

http://www.pharmainfo.net/majumdarshiv/ocular-drug-delivery-few-aspects
Cornea

- Cornea represents 7% of surface area of the eye.
- Cornea has a lower permeability for hydrophilic drugs than for lipophilic drugs.
- Only 2-5% of an ophthalmic drug can penetrate through cornea.

The cornea is 0.5 mm thick with three primary layers:
- Epithelium with thickness of 50 µm and 5-7 cell layers.
- Stroma is a 450 µm thick, porous and hydrophilic tissue.
- Endothelium a single layer in inner surface of the cornea.
Materials *In Vitro* Study

- Adult New Zealand white rabbit cornea: standard model for ocular drug delivery

- Unfocused custom-designed circular transducers (Sonic Concepts) with 15 mm active diameter at 400 kHz, 600 kHz, 800 kHz, and 1 MHz frequencies.

- The $d_{ff}$ calculated for these transducers are 1.5, 2.25, 3, and 3.75 cm respectively.
Drug Solutions

• **Sodium Fluorescein, 0.25%**
  – Used for diagnosis of corneal abrasions, corneal ulcers and infections
  – Hydrophilic
  – Maximum absorption @ 490 nm

• **Tobramycin, 0.3%**
  – Ophthalmic antibiotic formulation for tropical therapy of external infections
  – Hydrophilic
  – Maximum absorption @ 278 nm

• **Dexamethasone Sodium Phosphate, 0.1%**
  – Topical steroid solution used to suppress inflammatory response to different conditions
  – Hydrophilic
  – Maximum absorption @ 242 nm
Spherical Diffusion Cell

Spherical joint

9 mm

Sampling unit

Donor compartment

Receiver compartment

5 ml volume

http://www.permegear.com/franzatfaqs.htm
**In vitro Setup**

- Dissected cornea was placed over the spherical joint of diffusion cell, between donor and receiver compartments.

- Ultrasound was applied with intensities of 0.3 W/cm² - 1 W/cm² at different frequencies between 400 kHz - 1 MHz.
- The cornea was exposed to ultrasound for 5 min.
- Temperature was measured while applying ultrasound.
In vitro Setup

- The receiver compartment was stirred at 380 rpm using a magnetic stir bar.
- A 3 mL solution sample was collected through the sampling port of the receiver compartment after 60 min.
- The absorption of the sample was measured using a spectrophotometer.
- Dissected cornea was placed in formalin after the experiment to be fixed and sent for histology.
In vitro Histology

Different categories of histological damage are:

A. Class 0: None of the layers are damaged or missing (0).

B. Class 1: Some cells are missing or the first layer of epithelium is removed (1/3).

C. Class 2: Two layers are missing or damaged (2/3).

D. Class 3: All three layers are missing or epithelium is severely damaged (1).
Ultrasound application for 5 min at 1.0 W/cm² produced permeability increase of:

- 126% at 400 kHz (n=9),
- 121% at 600 kHz (n=13),
- 47% at 800 kHz (n=9),
- 65% at 1 MHz (n=12)

as compared to sham treated cases (n=9).
In vitro Results

The increase in corneal permeability ranged from 14% to 46.9% depending on ultrasound parameter combination, with no statistical significance achieved in all cases.
The percentage increase in corneal permeability to Dexamethasone Sodium Phosphate as compared to sham treated samples are shown in this table.
Changes in Corneal Epithelium *In vitro*

- Sham shows the corneal changes with no ultrasound treatment; different shades of gray represents the corneal damage due to ultrasound application.

- Data are shown as mean ± standard deviation.

* Indicates p-value < 0.05
** Indicates p-value < 0.01
*** Indicates p-value < 0.001
Experimental Preparation

• The most effective parameters used in vitro study
  – \( f = 400 \text{ kHz} \text{ and } 600 \text{ kHz} \)
  – Intensity = 0.8 \( \text{W/cm}^2 \)
  – Exposure time = 5 min
  – Total study time = 60 min

• Dexamethasone sodium phosphate

Driving unit of transducers

- Function Generator
- Amplifier
- Matching Network
- Unfocused Transducer
In vivo Setup

- The eye cup was placed on the eye filled with drug solution.
- Transducer was placed on a metal stand and submerged inside the solution.
- Ultrasound was applied with intensity of 0.8 W/cm² at different frequencies of 400 kHz and 600 kHz.
- The cornea was exposed to ultrasound for 5 min.
- Temperature was measured 3 times while applying ultrasound (t = 0, 2.5, and 5 min).
Methods

• After ultrasound application and also before euthanasia, *in vivo* gross observation of the cornea was performed using a high magnification stereomicroscope.

• About 0.3 mL sample of aqueous humor was collected using 27 G × 1/2" needle (12.7 mm length) approximately 60 min after the ultrasound treatment and immediately after the animal was euthanized.

• These samples were sent for chromatography.
Histological Analysis

- Thickness of different layers of cornea (epithelium, stroma, and endothelium).

Zeiss AxioImager light microscope at 5-20X magnification

- Investigating the structural changes in cornea using histology slides.
  - Same criteria used in *in vitro* study
Drug Concentration in Aqueous Humor

• Drug concentration in aqueous humor samples as compared to sham treated samples increased by:
  
  — 2.8 times using 400 kHz
  — 2.4 times using 600 kHz

• For sham treatments n=7, using 400 kHz frequency n=5, and n=6 using frequency of 600 kHz.
**In vivo Epithelial Change Comparison**

- The epithelial structural changes, observed in histological analysis, showed an increase of:
  - 4 times using 400 kHz
  - 3 times using 600 kHz

  ![Change in Corneal Epithelium Structure](chart)

  * Indicates p-value < 0.01
  ** Indicates p-value < 0.05

- For sham treatments n=8, using frequency of 400 kHz n=6, and n=6 using frequency of 600 kHz.
Drug Concentration vs Epithelial Damage

There is a direct relation between the drug concentration in aqueous humor and epithelial damage.
Temperature Changes

- The change in temperature from \( t = 0 \) to \( t = 5 \) min:
  - In ultrasound-treated cases was
    - 3 - 6 °C (4.0 ± 1.1 °C) for 400 kHz
    - 4 - 5 °C (4.8 °C ± 0.4°C) for 600 kHz
- Temperature recorded at different time intervals:

<table>
<thead>
<tr>
<th></th>
<th>Temp (t=0 min)</th>
<th>Temp (t=2.5min)</th>
<th>Temp (t=5min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-treated</td>
<td>25.3±0.7</td>
<td>25.6±1.0</td>
<td>26.6±1.3</td>
</tr>
<tr>
<td>Ultrasound-treated</td>
<td>25.3±1.0</td>
<td>27.8±1.3</td>
<td>29.7±0.1</td>
</tr>
</tbody>
</table>

Values are shown as mean ± standard deviation
Modeling Objectives

- Thermal effects in different parts of the eye
- Temperature increase at different parameters
- Validating *in vitro* and *in vivo* results for temperature increase in cornea
- Limitation: no perfusion
  - No blood flow in cornea and lens
- PZFlex
### Geometric Eye Model

Dimensions for rabbit and human eyeball structures in mm.

<table>
<thead>
<tr>
<th></th>
<th>Rabbit</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antero-posterior length</td>
<td>16-19</td>
<td>23-25</td>
</tr>
<tr>
<td>Anterior chamber depth</td>
<td>2.9</td>
<td>3.5</td>
</tr>
<tr>
<td>Thickness of Cornea in center</td>
<td>0.3-0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Diameter of Cornea</td>
<td>13.5-14</td>
<td>10.6</td>
</tr>
<tr>
<td>Thickness of Cornea in periphery</td>
<td>0.45</td>
<td>0.7</td>
</tr>
<tr>
<td>Thickness of Lens</td>
<td>6.36</td>
<td>3.5-4.3</td>
</tr>
<tr>
<td>Thickness of Sclera</td>
<td>0.328</td>
<td>0.5-1.0</td>
</tr>
<tr>
<td>Thickness of Choroid</td>
<td>0.068</td>
<td>0.1-0.5</td>
</tr>
<tr>
<td>Thickness of Retina</td>
<td>0.051</td>
<td>0.1-0.5</td>
</tr>
</tbody>
</table>

(Gwon 2008, Werner et al. 2006; Missel et al. 2010)
Acoustic and thermal characteristic of different eye structures

<table>
<thead>
<tr>
<th></th>
<th>Speed of Sound (m/s)</th>
<th>Acoustic Attenuation (dB/cm/MHz)</th>
<th>Specific Heat (J/kgK)</th>
<th>Thermal Conductivity (W/mK)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornea</td>
<td>1586</td>
<td>0.78</td>
<td>4178</td>
<td>0.58</td>
</tr>
<tr>
<td>Sclera</td>
<td>1647</td>
<td>0.97</td>
<td>4178</td>
<td>0.58</td>
</tr>
<tr>
<td>Aqueous humor</td>
<td>1497</td>
<td>0.01</td>
<td>3997</td>
<td>0.59</td>
</tr>
<tr>
<td>Choroid</td>
<td>1527</td>
<td>0.95</td>
<td>3840</td>
<td>0.60</td>
</tr>
<tr>
<td>Lens</td>
<td>1647</td>
<td>1.19</td>
<td>3000</td>
<td>0.40</td>
</tr>
<tr>
<td>Vitreous humor</td>
<td>1532</td>
<td>0.01</td>
<td>3999</td>
<td>0.60</td>
</tr>
<tr>
<td>Retina</td>
<td>1538</td>
<td>1.15</td>
<td>3680</td>
<td>0.57</td>
</tr>
<tr>
<td>Optical Nerve*</td>
<td>1644</td>
<td>0.7</td>
<td>3750</td>
<td>0.53</td>
</tr>
</tbody>
</table>

(Opie et al. 2010, Duck 1990, deKorte et al.1994)
Modeling Setup

- Theoretical model of whole eye based on accurate geometrical measurements, and acoustic and thermal characteristics of eye structures.
- An unfocused continuous ultrasound beam at frequency of 400 KHz - 1 MHz and 0.3-1.0 W/cm² intensities.
- Axi-symmetric modeling
- Base temperature = 37°C
Modeling Setup

- Unfocused transducer with 15 mm active diameter was placed at $d_{ff}$.
- The entire eye, other than cornea, was placed inside tissue mimicking gel.
- The material between eye and transducer was water.
Modeling Ultrasound Wave Propagation into Eye

\[ I = \frac{p_0^2}{2 \rho c} \rightarrow p_0 = \sqrt{I \times 2 \rho c} \]

\text{I = intensity } \text{W/m}^2, \text{ p}_0 \text{ is the pressure amplitude in kg/s}^2 \text{m, } \rho \text{ is density in kg/cm}^3, \text{ and } c \text{ is speed of sound in m/s.}
Results at 400 kHz

$T_{\text{MAX}} \sim 39^\circ\text{C}$ in the lens at frequency of 400 kHz and intensity of 1.0 W/cm$^2$. 
Results at 600 kHz

$T_{\text{MAX}} \sim 40.5^\circ\text{C}$ in the lens at frequency of 600 kHz and intensity of 1.0 W/cm$^2$. 
Results at 800 kHz

$T_{\text{MAX}} \sim 42.5^\circ \text{C}$ in the lens at frequency of 800 kHz and intensity of 1.0 W/cm$^2$. 
Results at 1 MHz

$T_{\text{MAX}} \sim 43.5^\circ C$ at proximity of the lens at frequency of 1 MHz and intensity of 1.0 W/cm$^2$. 
Future Work

• Investigating safety factors of ultrasound application in the proximity of the bone (for example optical nerve) and also bone itself.

• Using pulsing method may increase the treatment time but would result in lower temperature increase.

• Drug delivery into the back of the eye.

• Ocular delivery of macromolecules.
Conclusions

• Confirmed the use of ultrasound *in vitro* and *in vivo*, increased ocular drug delivery.
• Skills in tissue processing, animal handling, and image analysis using microscope were developed.
• A set up demonstrated the feasibility of mechanical and thermal effect of ultrasound in enhancement of corneal permeability.
• A model was established for safety of this application.

Acknowledgments

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