In Vitro and In Vivo Low Level Laser Therapy in the Gentamicin Induced Ototoxicity of the Rat Cochlea
Results: penetration (Rats)

Before Drilling: 165 mW → 6.15 mW (3.73% delivered)

After Drilling
• 165 mW → 11.36 mW (6.88% delivered)
  → 12.35 mW (7.48%) → 10.1 mW (6.12% )
  → 8.5 mW (5.15%) → 9.6 mW (5.82% )
  → 11.3 mW (6.85%) → 6.42 mW (3.89% )
  → 7.39 mW (4.48%) → 12.64 mW (7.66% )
  → 11.4 mW (6.91%) → 11.53 mW (6.99% )

Average Penetration → 10.24±2.04 mW (6.20±1.24% delivered)
Penetration (Human TB)

- Two cadaver temporal bones
  LLLT: 830 nm (80 mW) transcanal to tympanic membrane & cochlea wall

Result

- Penetration through TM: 4 mW (5%)
- Penetration through cochlea wall: 1.6 mW (2%)
Transmeatal LLL irradiation to Cochlea
Penetration rate: ~6%
200mW irradiation
H&E staining

Un-irradiated ear
Lt ear

Irradiated ear
Rt ear

Middle ear

External ear

Malleus

Malleus removed
<table>
<thead>
<tr>
<th></th>
<th>Un-irradiated ear</th>
<th>Irradiated ear</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tympamic membrane</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelial lining</td>
<td>Intact</td>
<td>Intact</td>
</tr>
<tr>
<td>Inflammation</td>
<td>Not identifiable</td>
<td>Not identifiable</td>
</tr>
<tr>
<td><strong>External ear</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelial lining</td>
<td>Intact</td>
<td>Intact</td>
</tr>
<tr>
<td>Sebaceous gland</td>
<td>Intact</td>
<td>Intact</td>
</tr>
<tr>
<td>Inflammation</td>
<td>Not identifiable</td>
<td>Not identifiable</td>
</tr>
<tr>
<td>Vasculature</td>
<td>Normal</td>
<td>Engorged</td>
</tr>
</tbody>
</table>
Our results show that 830nm laser penetrated tympanic membrane and cochlea bone by 2 % in human & 6 % in rat.

There has not been any side effects at external ear canal, ear drum, and middle ear except hyperemia of external canal skin and drum.
The effect of 830nm laser on inner ear hair cell

- To evaluate the adverse effects of 830nm LLLT on cochlear hair cells and hearing level.
Method

- Guinea pigs & 830 nm LD were used for this study.
- Right ear: 100 mW/cm² × 30 minutes (180 J/cm²) irradiation with laser tip into ear canal towards ear drum 5 ds/wk for 2 wk
- Left ear: control – no laser irradiation
- Hearing test by ABR on 7th, 10th, and 14th day
- On 14th day, temporal bones were harvested for
- Cochlear hair cells were examined by SEM.
Hearing threshold (8kHz)

Result
SEM

control

laser
SEM

control

laser
Conclusion

- 830nm (180 J/cm²) laser has no adverse effects on hearing and cochlear hair cells in guinea pigs.
Aim

• The effects of LLLT on cochlear hair cell regeneration following gentamicin ototoxicity.
  – In vitro: organ culture of rat cochlea
  – In vivo: functional effectiveness in rat
In vitro study

- An organotypic culture of 5-day old SD rat cochlea
- 4-5 animals (7-8 cochlea) for each group.
  - Control group (C group): in Dulbecco’s phosphate Buffered saline, (DPBS) and not exposed to gentamicin nor laser
  - Laser only group (L group):
    - not exposed to gentamicin but irradiated daily (12d)
  - Gentamicin group (G group):
    - exposed to 1 mM of gentamicin for 48 hr
  - Gentamicin+Laser group (GL group):
    - exposed to 1 mM of gentamicin for 48 hr and then irradiated daily (12d)
Laser setting

- LLLT (808 nm, T&L, Daejeon, Korea) was used to irradiate the samples once a day at 8 mW/cm² x 60min (28.8 J/cm²) at room temperature. The distance from the fiber optic tip to the sample was approximately 50 cm.
Hair cell staining

- FM 1-43 was added directly to culture media
  - for 5 min (final concentration, 2µg/ml),
  - confocal microscope (LSM510 META, Zeiss)

- FM1-43 [N-(3-triethylammonium-propyl)-4-(4-dibutylamono)-styryl) pyridinum dibromide]
  - widely used to achieve synaptic vesicle recycling.
  - non-toxic, fluorescent, cationic dye
  - stains the cytoplasm of hair cells,
Hair cell counting

- Two steps of preprocessing: adjust the difference of staining intensity and microscope gain.
  - First, density calibration
  - Then, density slicing
  - Only the signals which were larger than 6 µm were considered as a hair cell.

Original images were changed into gray scale.
Density calibration was done, so that the density of the HC is 255 and that of the background is 0 in all the images (Photoshop).
Density slicing was done, so that signal weaker than 110 will be erased (Scion Image).
Signals stronger than 110 and larger than 6 µm were assumed as live HCs and were counted.
Control and Laser only group

- The RM ANOVA for the number of hair cells revealed a significant effect for group ($p=0.05$). And the group $\times$ time interaction was also significant ($p=0.04$). That is, the number of hair cells was significantly larger in the Laser only group. Also, the number of hair cells in the Control group showed decreasing tendency (steeper inclination) which was significantly different from the Laser only group. (N: no of animal, n: no of cochlea)
The RM ANOVA for the number of hair cells revealed a significant effect for group \((p=0.01)\). And the group \(\times\) time interaction was also significant \((p=0.01)\). That is, the number of hair cells was significantly larger in the GM + Laser group. Also, the number of hair cells in the GM + Laser group showed increasing tendency (steeper inclination) which was significantly different from the G group. (N: no of animal, n: no of cochlea)
COCHLEA HAIR CELL RESCUE AFTER NOISE-INDUCED HEARING LOSS (NIHL) USING LOW LEVEL LASER IN VIVO

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\textsuperscript{2}DEPARTMENT OF OTOLARYNGOLOGY-HNS DANKOOK UNIVERSITY, CHEONAN, KOREA
Before noise exposure (n=11, 11)

threshold (dB SPL)
frequency (kHz)

- AB R, before noise
Results: ABR Recordings

ABR after 1st Laser (n=11, 11)

ABR after 5th Laser (n=11, 11)

ABR after 10th Laser (n=11, 11)

ABR after 12th Laser (n=11, 11)
Results: ABR Recordings

Before noise exposure (n=11, 11)

![Graph showing ABR recordings before noise exposure.](image)

ABR after 12th Laser (n=11, 11)

![Graph showing ABR recordings after 12th Laser treatment.](image)
### SEM study (hair cells counts)

<table>
<thead>
<tr>
<th></th>
<th>Apex (cells)</th>
<th>Middle (cells)</th>
<th>basal turns (cells)</th>
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<tbody>
<tr>
<td><strong>Control group</strong></td>
<td></td>
<td></td>
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<tr>
<td>(n=6)</td>
<td>176.5±16.3</td>
<td>146±5.6</td>
<td>154.8±6.7</td>
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<tr>
<td><strong>Noise Rt ear</strong></td>
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<tr>
<td>(n=16)</td>
<td>122.5±2.1*</td>
<td>113.5±2.1*</td>
<td>133.3±7.6*</td>
</tr>
<tr>
<td><strong>Laser Lt ear</strong></td>
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<tr>
<td>(n=16)</td>
<td>167.4±27.5</td>
<td>140.4±29.7</td>
<td>147±13.8</td>
</tr>
</tbody>
</table>
Conclusion

• This study has demonstrated that the immediate LLLT promoted restoration of NIHL.

• LLLT appeared to inhibit or reverse apoptotic process of hair cells by noise leading to hearing loss.

• LLLT may have clinical implications to treat various vestibular and cochlear inner ear diseases.