“Neural stem cells for Hearing Impairment.”

The Cochlea


Hearing Impairment

Abnormalities in the hair cells of the organ of Corti in the cochlea

The Cochlea: Avian & Mammalian

Jongkamonwiwat et al., CDT 2010

Ronaghi et al., Stem Cells 2011
The Cochlea: Avian & Mammalian

Hair cell from ESCs & iPSCs

The routes of hair cell transplantation

Cochlear Implant

Surgically implanted electronic device that provides a sense of sound. Directly stimulating any functioning auditory nerve inside the cochlea with an electric field.

People with mild or moderate sensorineural hearing loss are generally not candidates for cochlear implantation.

LETTER

Restoration of auditory evoked responses by human ES-cell-derived otic progenitors

Adult Stem Cell

Demonstrated success in some treatments

May be genetically matched to patient

Produce limited number of cell types

Not found in all tissue

Difficult to identify, isolate, maintain and grow in laboratory

Ethical concern: Destruction of human blastocyst

ESCs

Can produce all cell types

Relatively easy to maintain & grow

Risk of creating teratomas (tumours) from implanting undifferentiated cell

Ethical concern: Processes involved with retroviruses

iPSCs

Genetically matched to patient

Can produce all cell types

Processes involved with retroviruses

Long-term transplantation effects has not been proven

People with mild or moderate sensorineural hearing loss are generally not candidates for cochlear implantation.
Overall View
- hESCs Induction
- Characterization of Induced Cells
- Maintain of Induced Cells

Induction of Neuroprogenitor Phenotypes from hESCs
- Harvested by trypsinization
- Mechanical Selection
- Enzyme Free

Day 0
- GFP
- FGF 3 & FGF 10 Induction
- Maintain in OSCFM

Day 1
- ONPs

Day 10
- ONPs

Chen et al., Stem cell, 2009

Differentiation of apical structure and proto hair bundle in hair cell-like cells

Electrophysiological properties of differentiating hair cell-like cells and neurons

Chen & Jongkamonweat et al., Nature 2012
**Induced SHEF1-GFP: Early Stage**

Chen & Jongkamonwiwat et al., Nature 2012

**Induced SHEF1-GFP: Neuralizing Media**

Harvested by trypsinization

Ouabain: Na⁺/K⁺ ATPase Inhibitor

Ouabain induces apoptotic cell death in type I spiral ganglion neurons.

Lang H. et al., 2005

Application Dose: 20μl of 1mM Ouabain (Sigma,O-3125) in NSS

Applied to RW membrane for 30 minutes

Removed by small piece of cotton wick

Lang H. et al., 2008

**Deafness induction and Cell transplantation**

Comparison between Ouabain Treated and Untreated Cochlea

Comparison between Ouabain Treated and Untreated Cochlea
**Persistence of Hair cells in Ouabain treated cochlea**

Ouabain induces apoptotic cell death in type I spiral ganglion neurons.

**Deafness induction and Cell transplantation**

- A Nanoliter Microinjection System (WPI) with 33gauge needle
- 3 μl (~5x10⁴) of cell suspension in DMEM store at 4°C
- Daily injection of cyclosporine (15 mg/kg s.c.) starting 1 day before surgery and terminating the day before sacrifice.

**Immunohistochemistry Staining after Transplantation**

- RFP+ GluA2 NKA α3
- Neuron reorganization at the level of brainstem auditory nucleus
Auditory brainstem responses (ABR) and Distortion product otoacoustic emissions (DPOAE) have been applied to the evaluation of peripheral auditory function.

The purpose of this study was to measure click evoked ABR testing and 2f1-f2 DPOAE as detectors of sensorineural damage induced by ouabain treatment and also trace the recovery after transplantation.

Waveform nomenclature

Wave I: The wave occurring at 1.2 ms
Waves ii and iii: The complex double-waves at 2-3.5 ms
Wave iv: The large wave at 4.5 ms

ABR Threshold:
Stimulus level that evoked a peak-to-peak voltage 2 S.D. above the mean background activity.
Mean Background = 0.31 µV
S.D. = 0.08
2 S.D. = 0.16

Mean Background = 0.27 µV
S.D. = 0.09
2 S.D. = 0.18

Hearing Functional Test

Immunohistochemistry Staining after Transplantation

Hearing Measurement

Immunohistochemistry Staining after Transplantation

Click Level (dB SPL)

Burkard et al. (1993)
Transplanted cells Quantification

<table>
<thead>
<tr>
<th>Untransplanted ear (right)</th>
<th>Osseointegrated ear (left)</th>
<th>Surviving counts (x10⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21.5±1.2</td>
<td>24.3±2.0</td>
<td>51.7±2.8</td>
</tr>
<tr>
<td>23.1±1.4</td>
<td>25.8±2.2</td>
<td>50.9±2.0</td>
</tr>
</tbody>
</table>

From 5x10⁶ transplanted cells

Factors influence transplantation

The goal of optimizing these variables is to maximize the number of surviving functional cells present at the appropriate site of injury.

- **Timing**
- **Niche**
- **Administration route**
- **Immunosuppression**
- **Predifferentiation**
- **Pretreatment**
- **Scaffolds**
- **Controls**

Acknowledgements

This work was supported by grants from the RNID to Marcelo Rivolta.

Marcelo Rivolta
Wei Chen
Objoon Trachoo
Amanda Naylor
Sarah Jacob Eshtan

Faculty of Health Science, Srinakharinwirot University, Thailand
How do transplanted cells work?

1. Neurotransmitters released from the graft tissue.
2. Release of the neurotrophic/growth factors (BDNF, glial derived neurotrophic factor [GDNF], NGF, etc.) acting as local pumps to support cell function and to prevent cascade of apoptosis.
3. Regenerating neuronal population further prevents subsequent cell death.
4. Re-establishment of local interneuronal connections and synaptic connectivity between the host and graft.
5. Limit glial reaction and prevent retrograde degeneration.
6. Improvement of regional oxygen tension.
7. Cell differentiation and integration.
It is important that we temper any overly optimistic predictions as many patients and families already have unrealistic hopes of what can be achieved with cell-based and 'stem cell' therapies.