FUTURE THERAPIES FOR INNER EAR REGENERATION

Albert Edge
Harvard Medical School
Massachusetts Eye and Ear Infirmary

Hearing loss epidemic

One in ten (30 million) Americans has hearing loss

- Causes include heredity, aging, noise exposure, disease
- Number is expected to double by 2030

Hearing loss is the #1 birth defect in America

- 1 in 1000 newborns is born profoundly deaf
- 2-3/1000 will have partial/progressive hearing loss

Hearing loss prevalence increases with age

- 1 in 3 over 65 years has significant hearing loss
- Among seniors, hearing loss is the 3rd most prevalent condition

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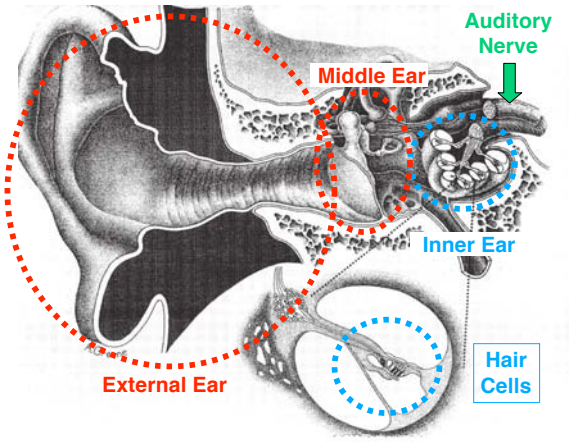
The inner ear



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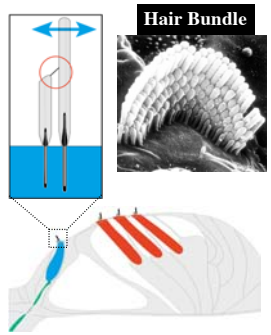
The hair cell

Sensory hairs vibrate, "tip-links"open ion channels into hair cell

Ions flow into hair cell, changing its electrical potential



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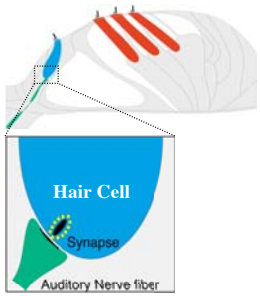
The nerve fiber

Electric potential causes chemical neurotransmitter release from synapse

Neurotransmitter diffuses to nerve fiber and excites electrical activity in the form of action potentials



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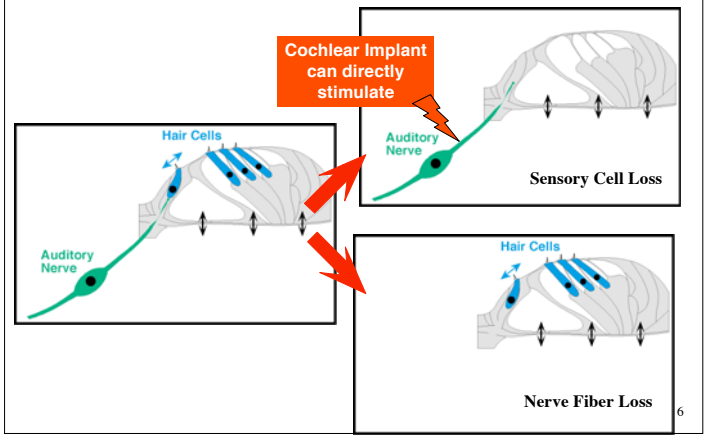
Sensorineural hearing loss:
Hair cells and nerve fibers



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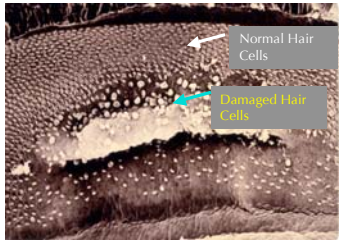
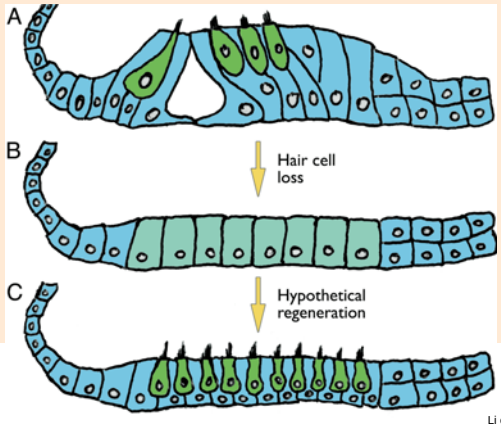
Regeneration of hair cells in chick inner ear



Can stem cell-derived inner ear progenitors replace lost hair cells *in vivo* (and restore hearing)?



Li et al., TMM (2004)

Approaches to regenerating inner ear cells

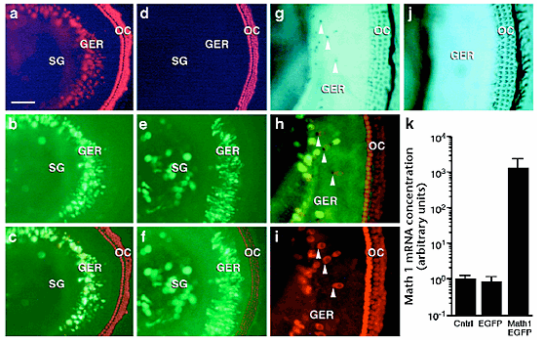
I. Generation of inner ear cells by gene therapy

II. Generation of inner ear cells from stem cells
- in vitro followed by cell grafting
- from endogenous cells in vivo

Gene therapy

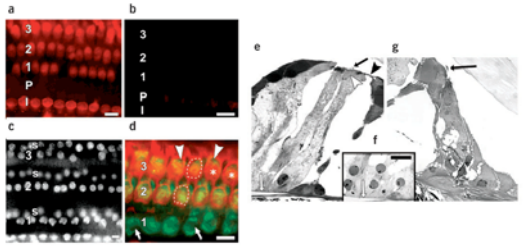
- New hair cells: transfer Atoh1 gene
- Cell division of existing hair cells: silence Rb gene

Transfection of Atoh1 leads to new hair cells



Zheng et al

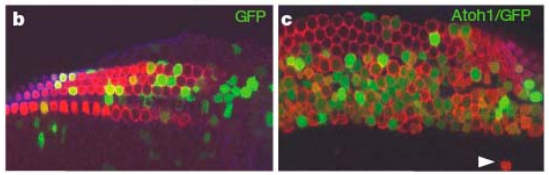
Overexpression of Atoh1 and formation of new hair cells



Izumikawa et al

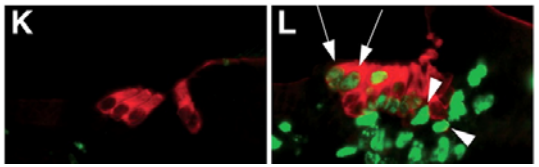
Gene transfer of Atoh1 during development leads to new hair cell formation

No gene transferred | Gene transferred



Silencing of gene that controls cell division leads to new hair cells

Normal cochlea | Silencing of regulator



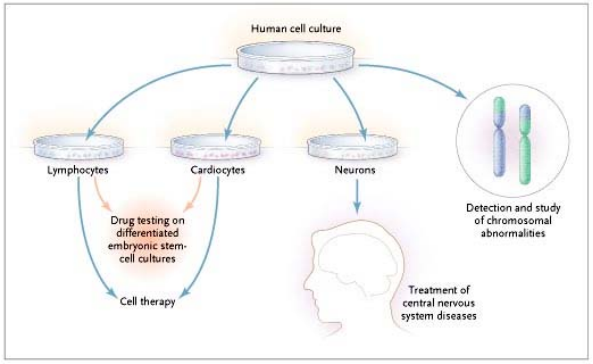
Generate new hair cells from stem cells

- Approach 1:
  - Generation of inner ear cell types from stem cells in a dish
  - Injection of hair cells or neurons into the inner ear
- Approach 2:
  - Generation of hair cells or neurons from stem cells in the animal

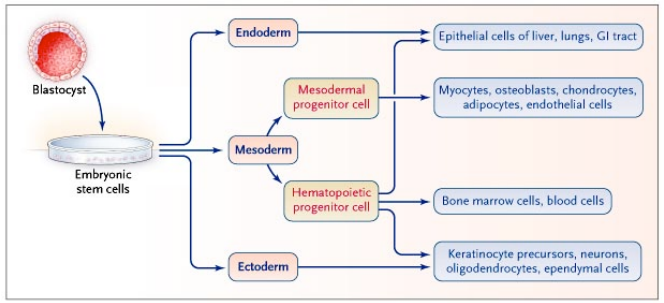
Generate new hair cells from stem cells

Approach 1: Cells from exogenous sources

- Generate hair cells and neurons from exogenous stem cells
- Transplant cells into the inner ear

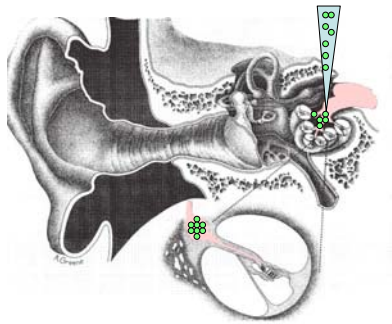


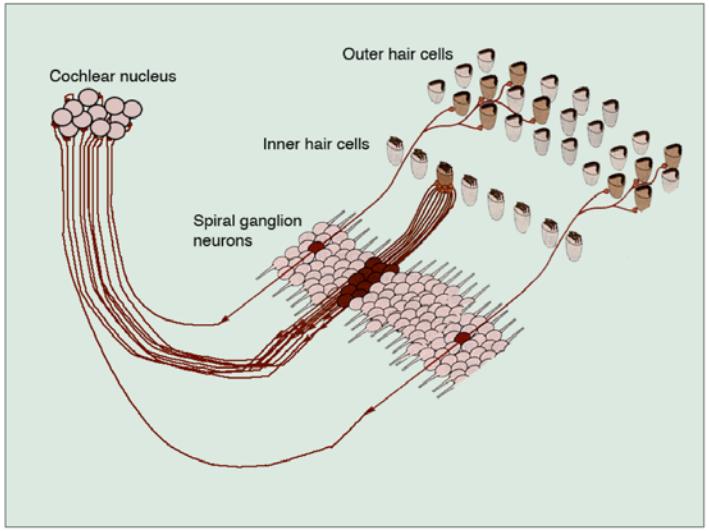
Embryonic Stem Cells

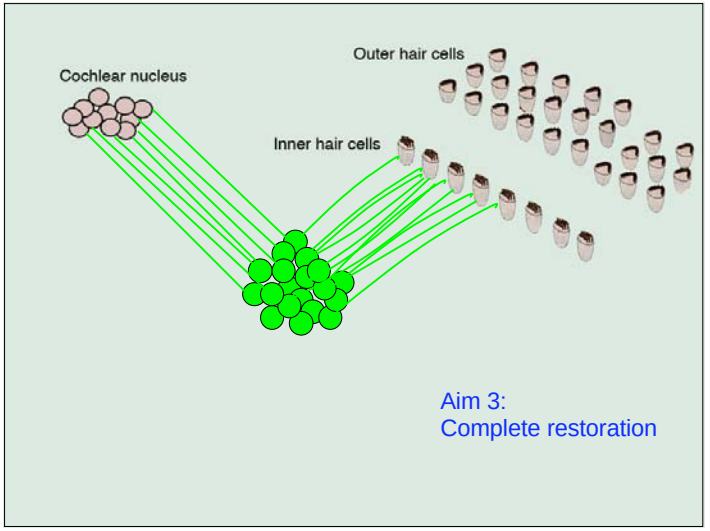


Adult Stem Cells

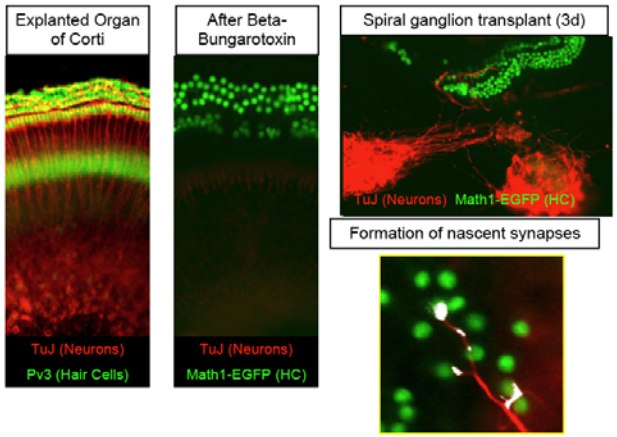
- Pluripotent (numerous cell types)
- Renewable
- No political issues for research use
- Avoid rejection problem because of autologous cells (from the same person)







Grafting into de-afferented organ of Corti explants



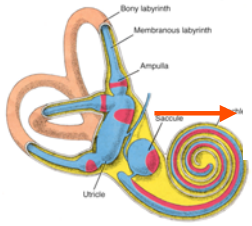
Generate new hair cells from stem cells

Approach 2: Activation of inner ear stem cells

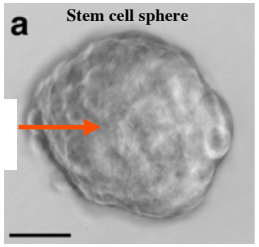
Can we activate genes that will lead to new hair cell or neuron formation?

Rebuilding the Human Inner Ear

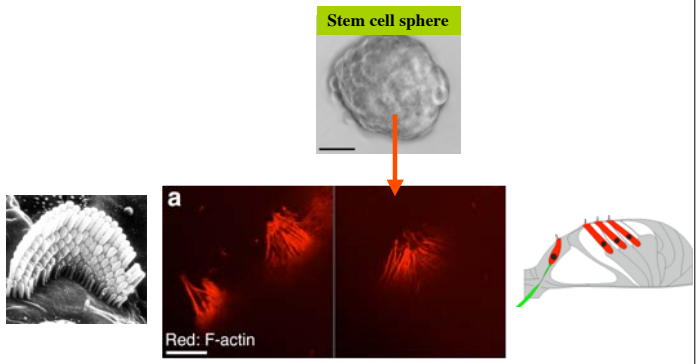
MEEI research team finds stem cells in mammalian ears



Stem cells from adult mouse and human inner ear



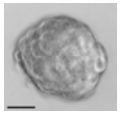
Rebuilding the Human Inner Ear

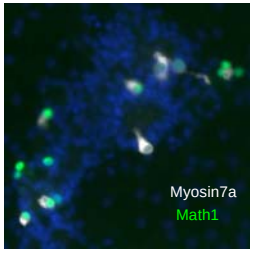


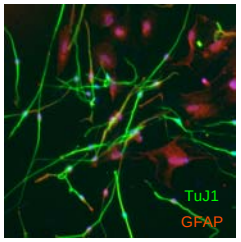
Hair Cells in a Dish

Differentiation into hair cells

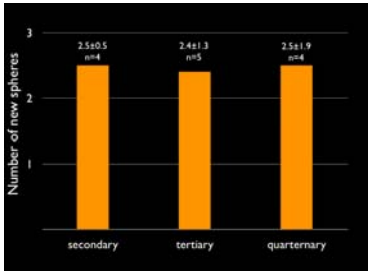
Differentiation into neurons and glial cells

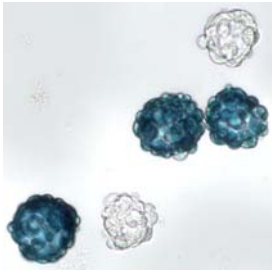






Spheres form from individual stem cells and self-renew with a renewal rate of 2-3





Mixed single cell suspensions from ROSA26 mice and C57/BL6 mice do not form mixed spheres

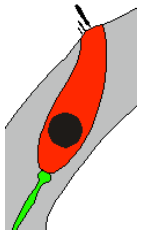
Identify compounds that increase hair cell formation

Strategy: Look for compounds that activate Atoh1

progenitor cell → hair cell

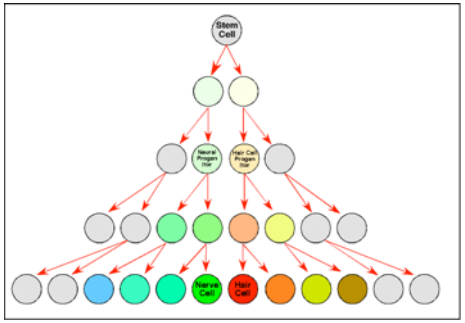
Luciferase under *Atoh1* promoter

No signal *Positive for luciferase*



Find a drug that increases the yield of hair cells

Gene switches in the pathway turned on or off by small molecules

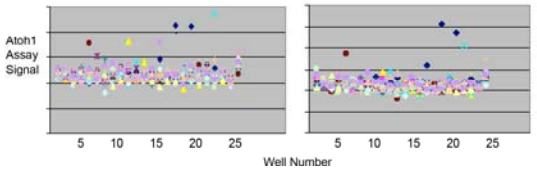


HARVARD Medical School SCREENING FACILITY

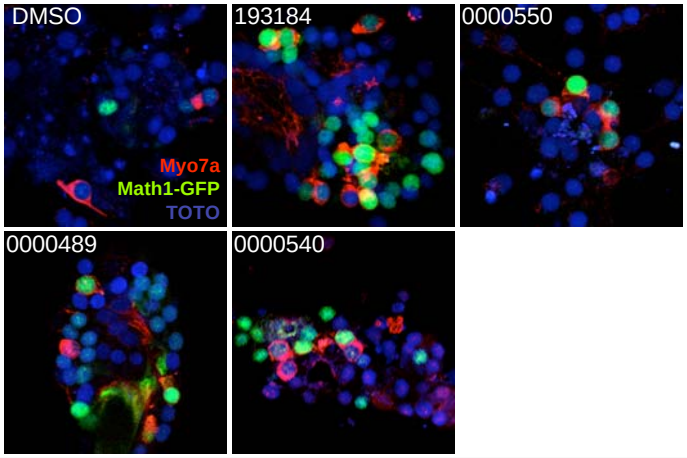
Drug screening facility

- Facility is well equipped
- Protocol approved for use of libraries and equipment

Screening Atoh1 reporter cells



Compounds increase hair cell differentiation from inner ear derived stem cells



Getting drugs to their targets: systemic vs. local delivery

For therapies based on these discoveries to become clinically useful, need to develop safe and reliable methods for delivery of complex compounds ***directly*** into the inner ear.

Challenges for drug delivery into the cochlea

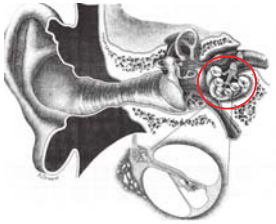
Cochlea is protected from most drugs applied to the bloodstream.

Cochlear fluid space is very small and sensitive to changes in fluid volume.

Useful drugs may be unstable over long periods of time in solution.

Frequent drug refills may introduce bacterial contamination.

Better hearing through chemistry: inner ear drug delivery



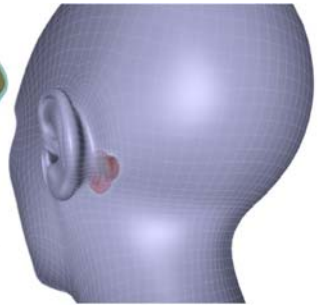
Blood-Cochlea Barrier:

Prevents ready access to cochlea (good and bad)

Future therapies based on complex compounds will benefit from ***direct*** intracochlear delivery

Rebuilding the human inner ear

Working with MIT engineers at Draper Lab



Our Vision

-a small implantable device to deliver drugs to the ear for several years

-a reservoir of dried, concentrated, stabilized drug

-timed, sequenced release of multiple drugs under microprocessor control.