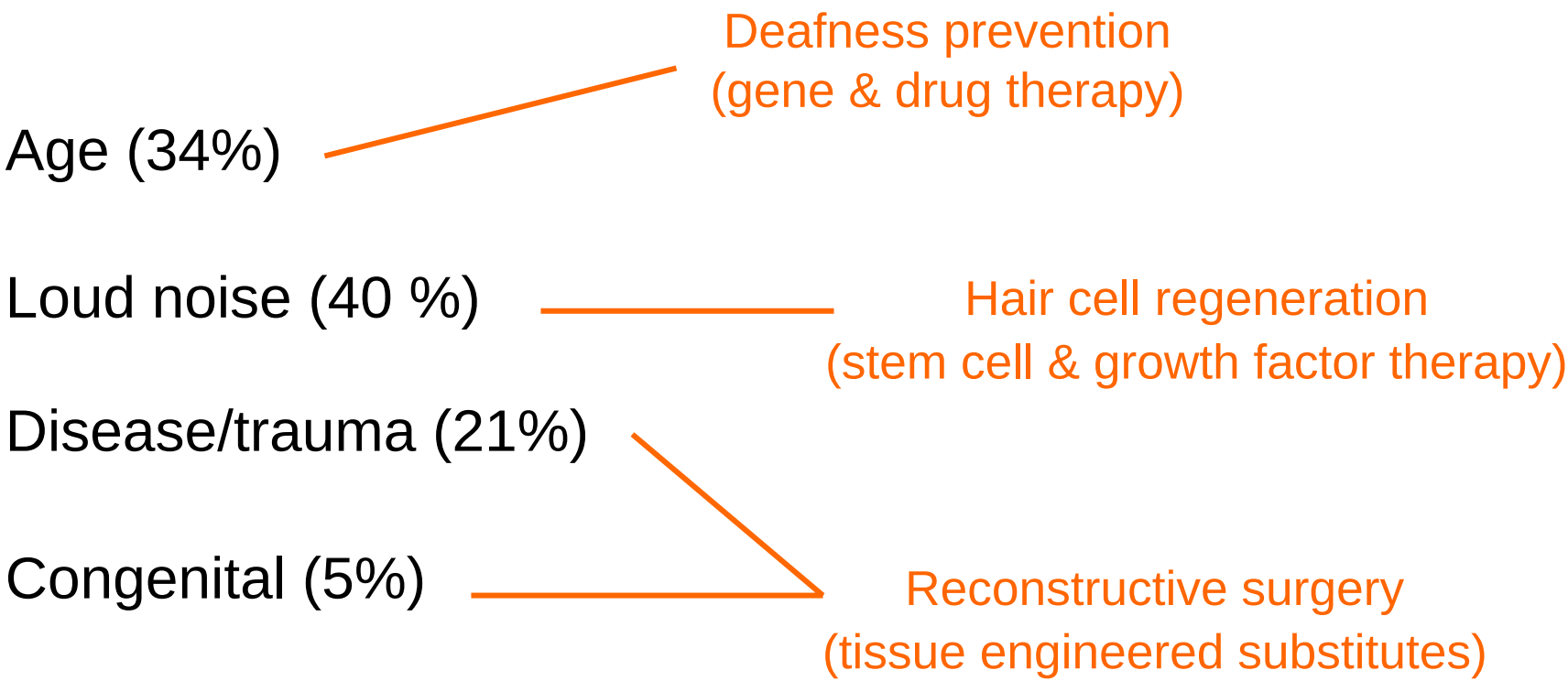
Cell and Tissue-based Therapies for Hearing Loss

Anne L. van de Ven

19 Nov 2003

Causes of Hearing Loss

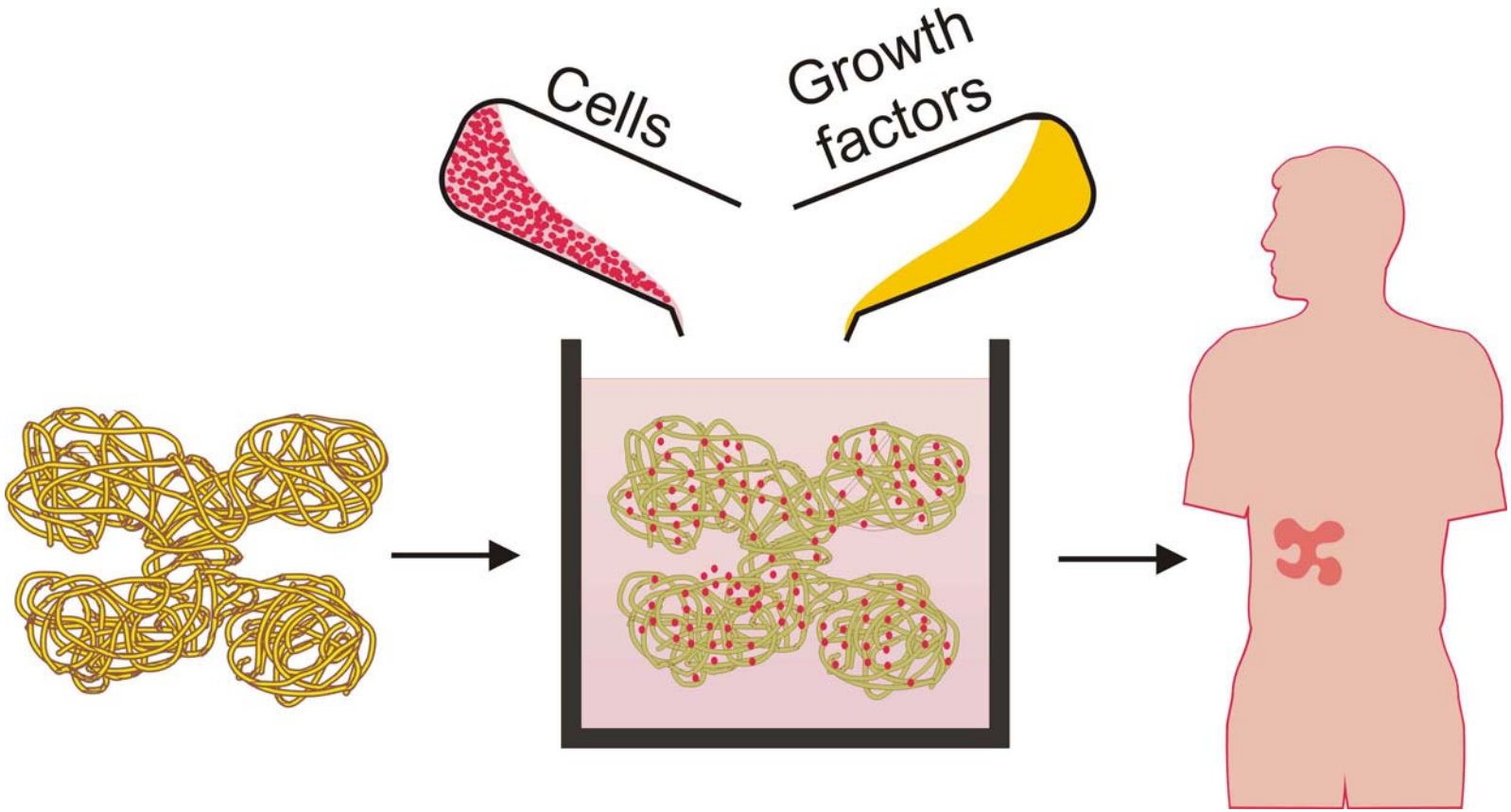


Each requires different strategies for treatment

Tissue Engineering

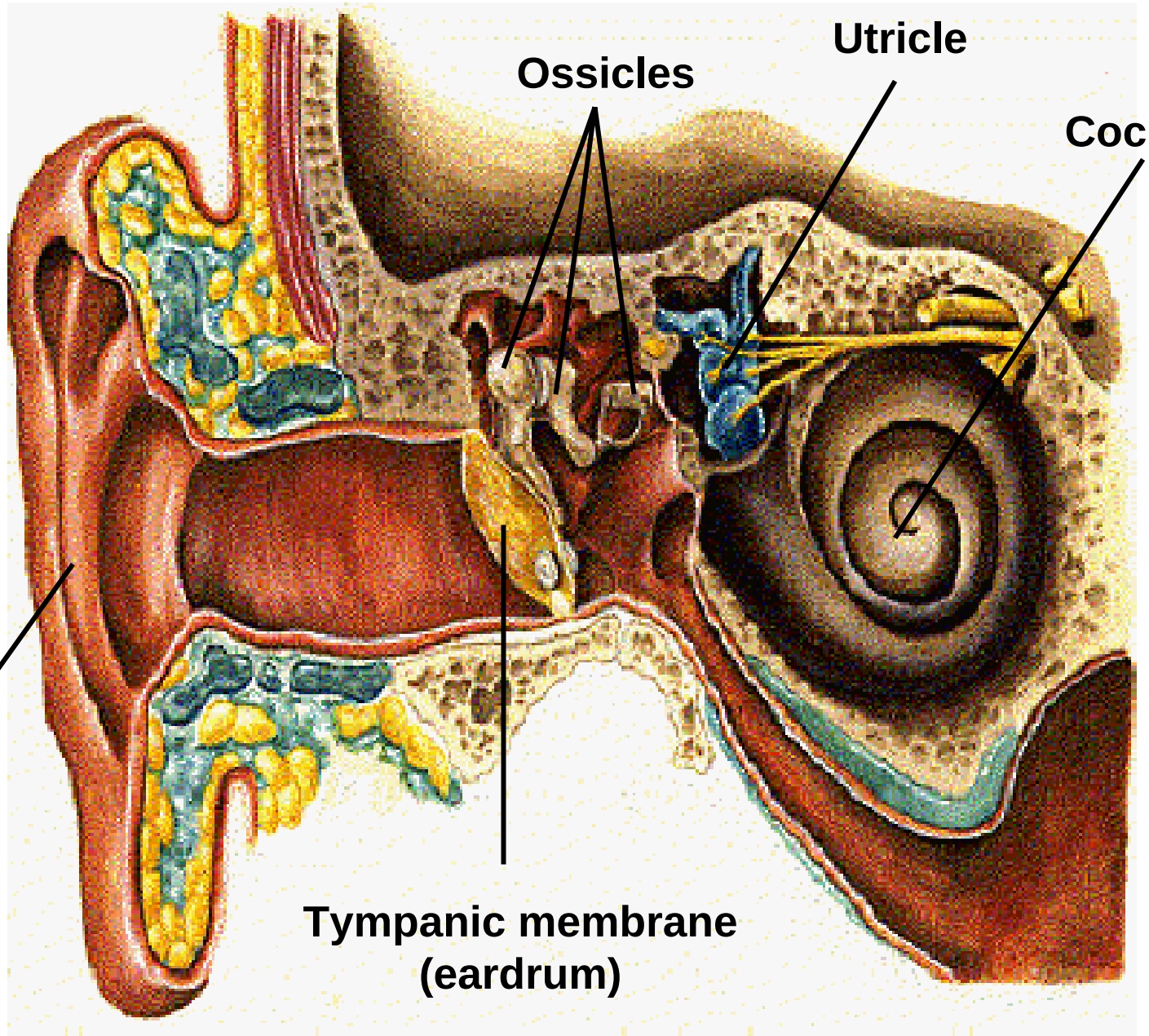
Repair or replacement of tissue function with biological materials generated in labs

Tissue Engineering



Tissue Engineering of the Ear

- Cochlear regeneration
- Outer ear reconstruction
- Tympanic membrane repair
- Middle ear ossiculoplasty


Utricle
Ossicles
Cochlea
Auricle
Tympanic membrane
(eardrum)

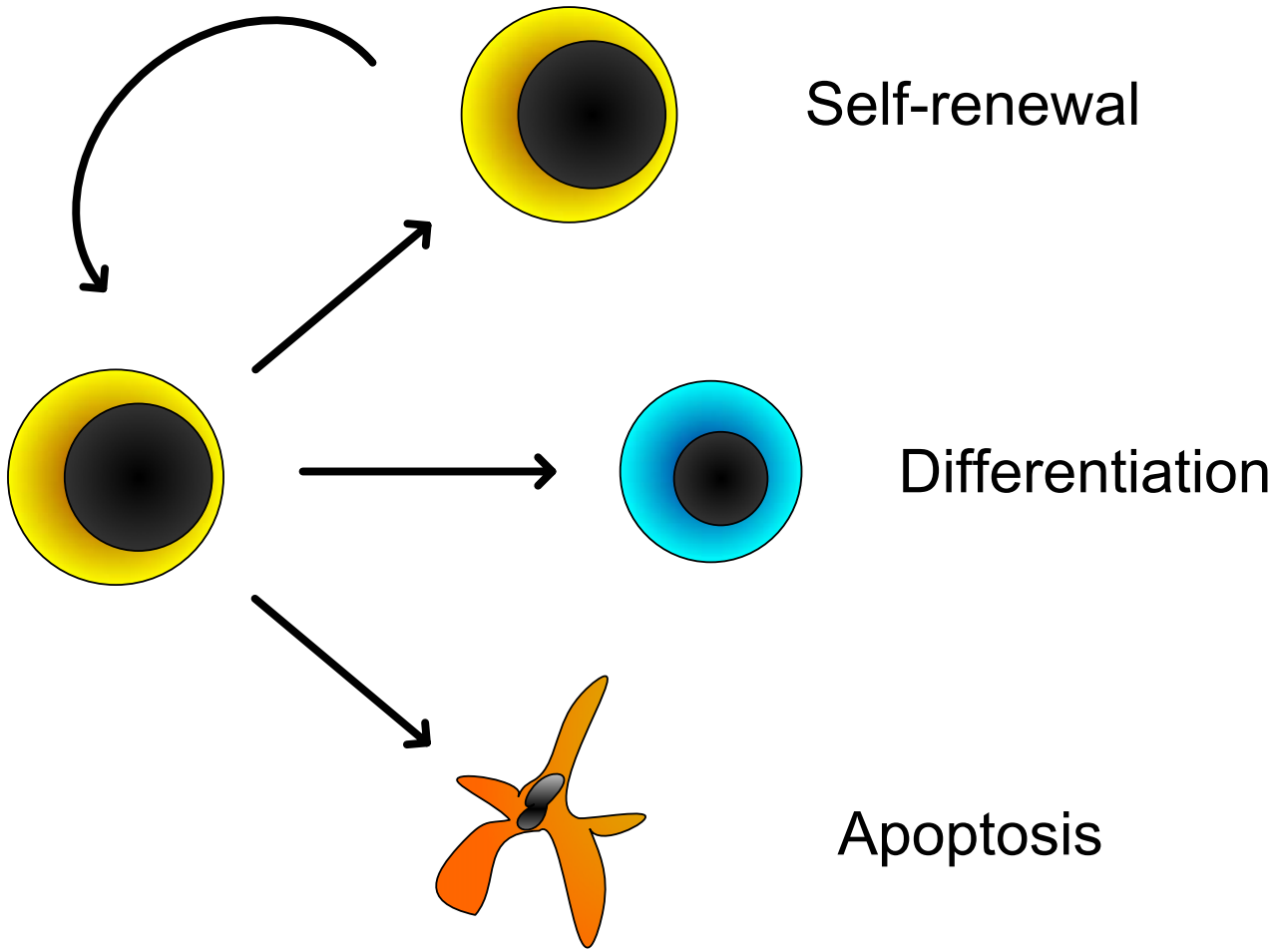
Tissue Engineering in the Cochlea

Aimed at regenerating/replacing damaged hair cells

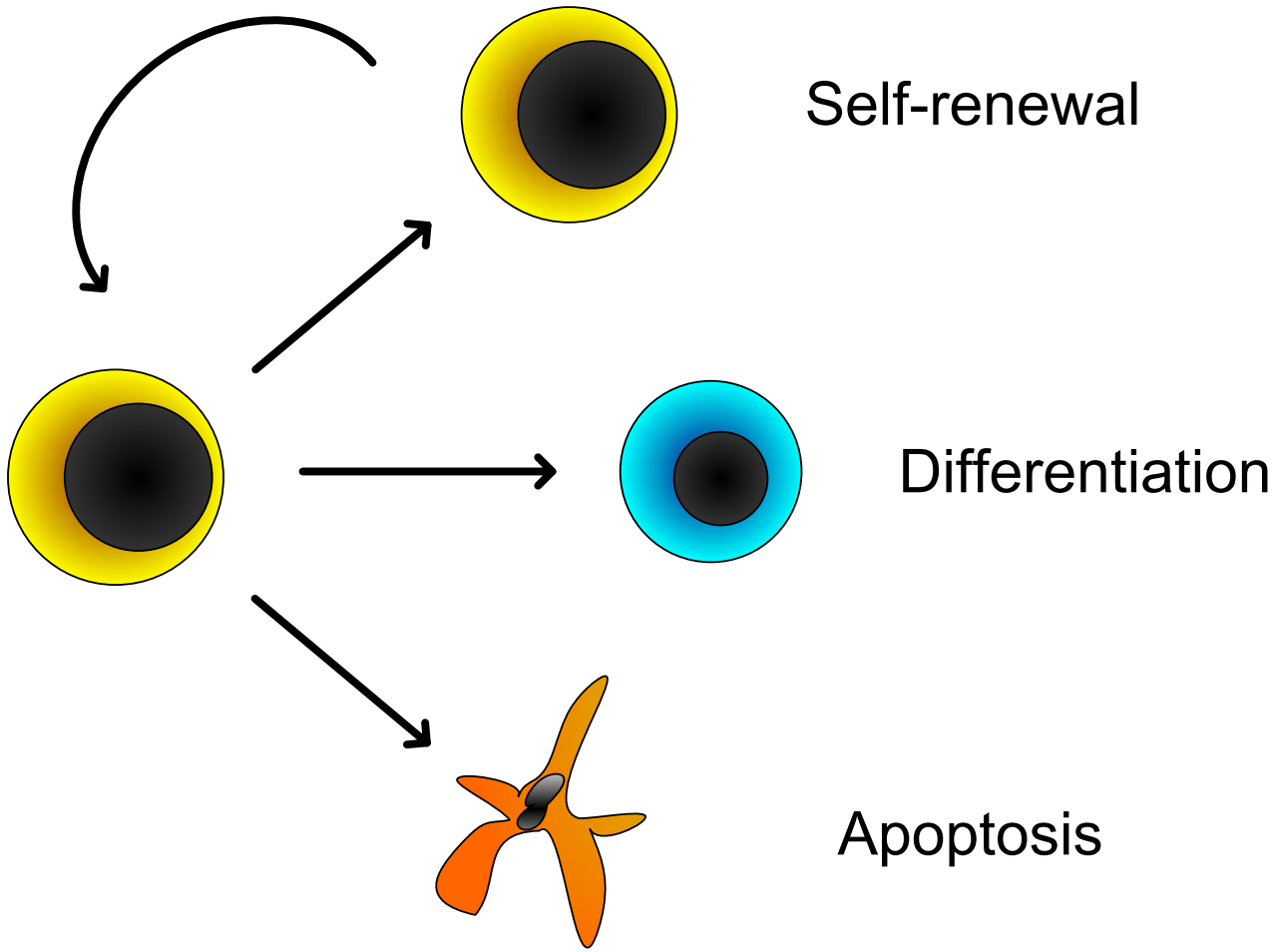
Potential cell sources:

- Embryonic stem (ES) or progenitor cells
- Adult inner ear stem cells
- Trans-differentiation of non-hair cells

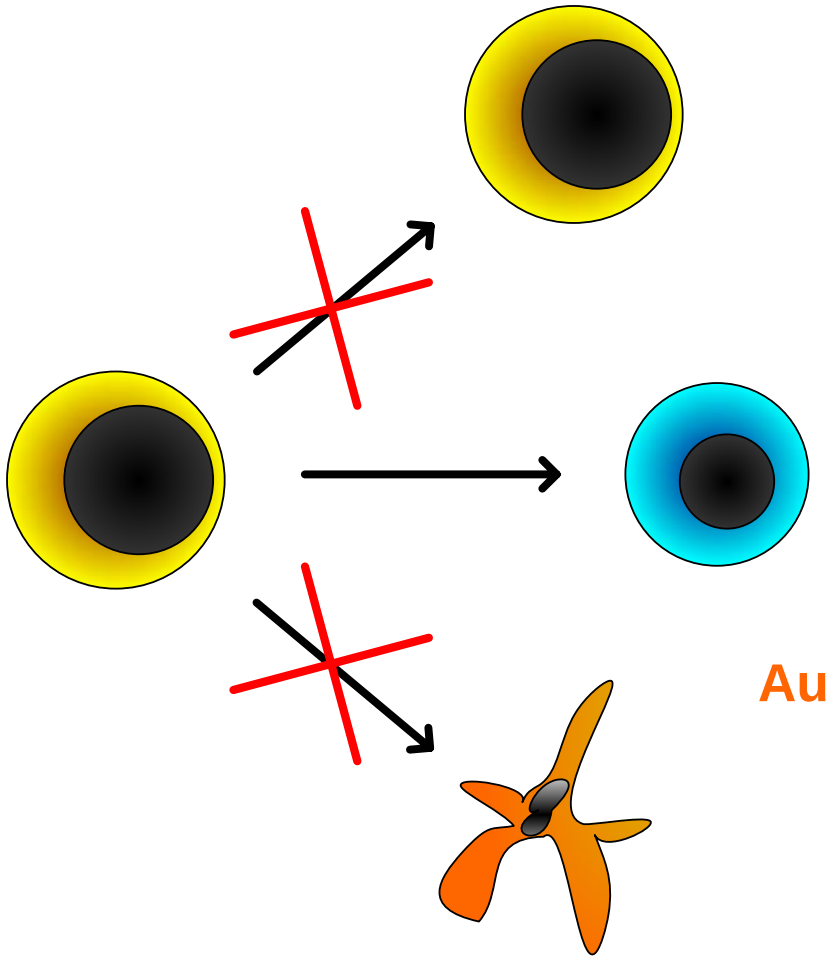
Stem Cell Fate



Stem Cell Fate



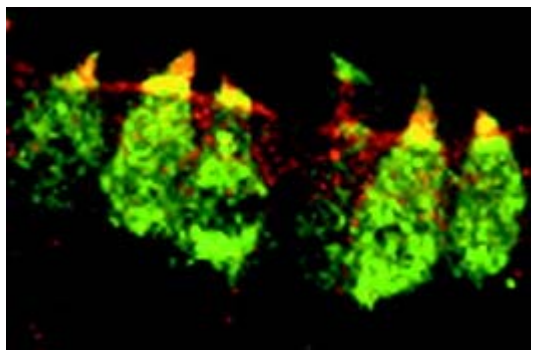
Stem Cell Fate



Differentiation

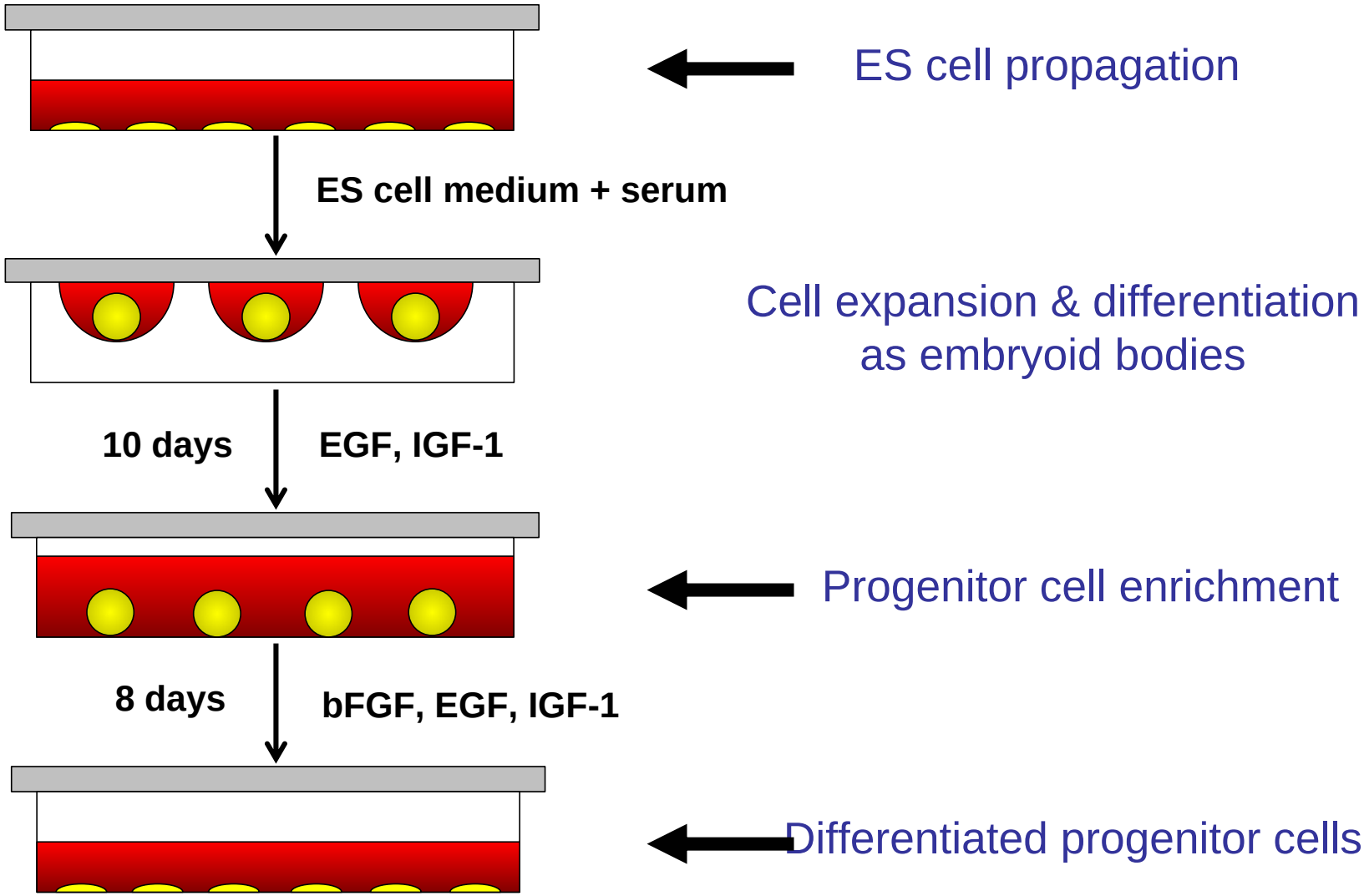
Auditory progenitor cells

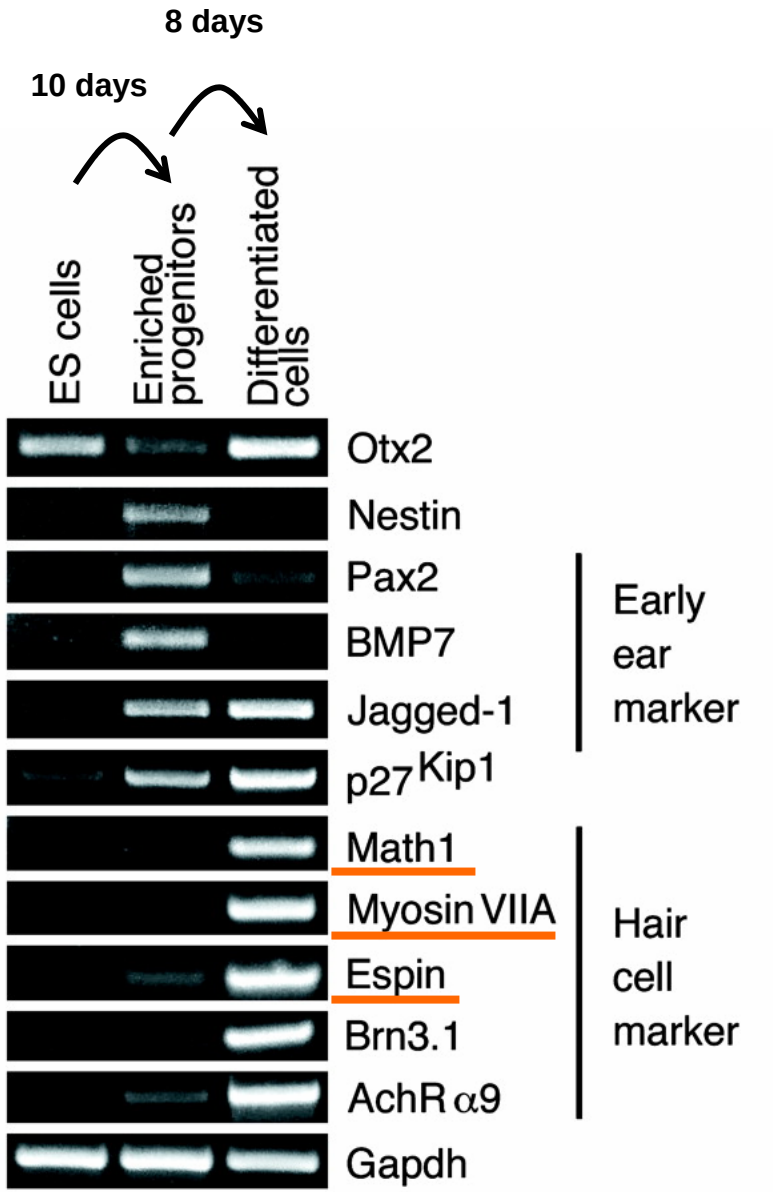
Generation of hair cells by stepwise differentiation of embryonic stem cells

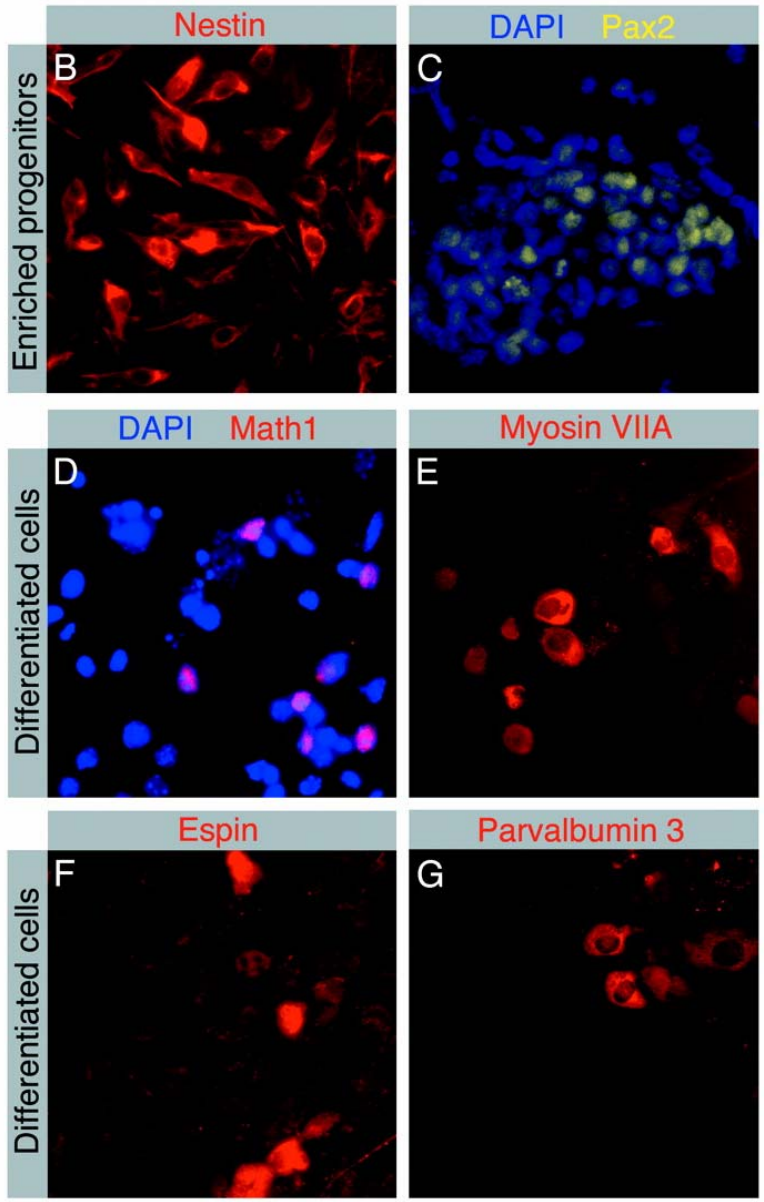


Li H,Roblin G, Liu H, Heller S.

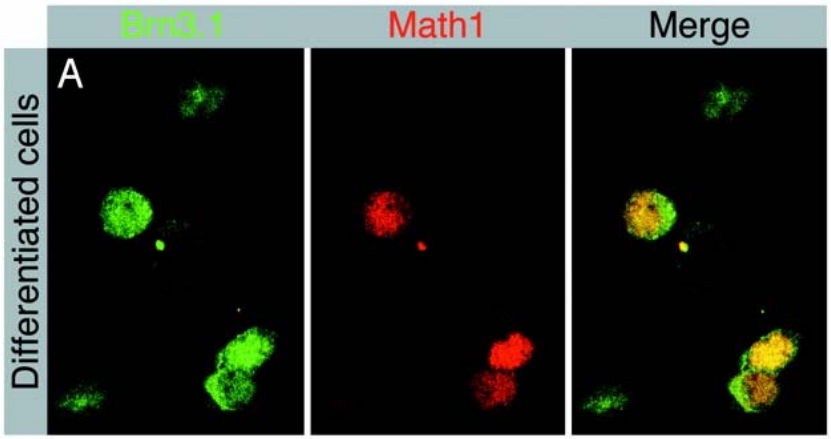
Creating Auditory Progenitor Cells

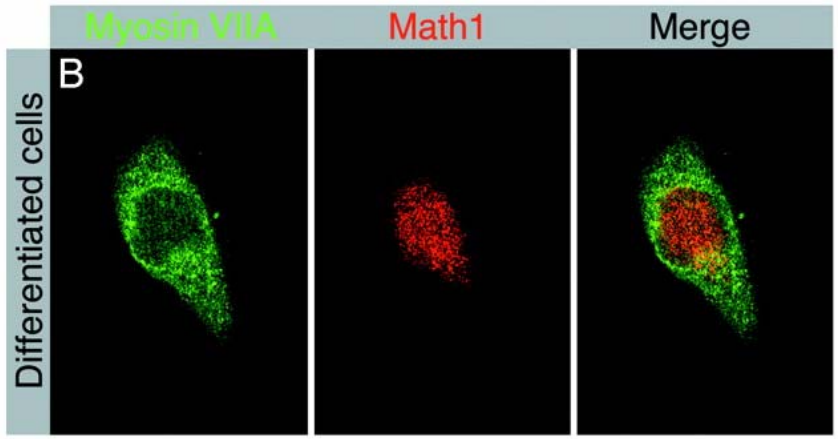


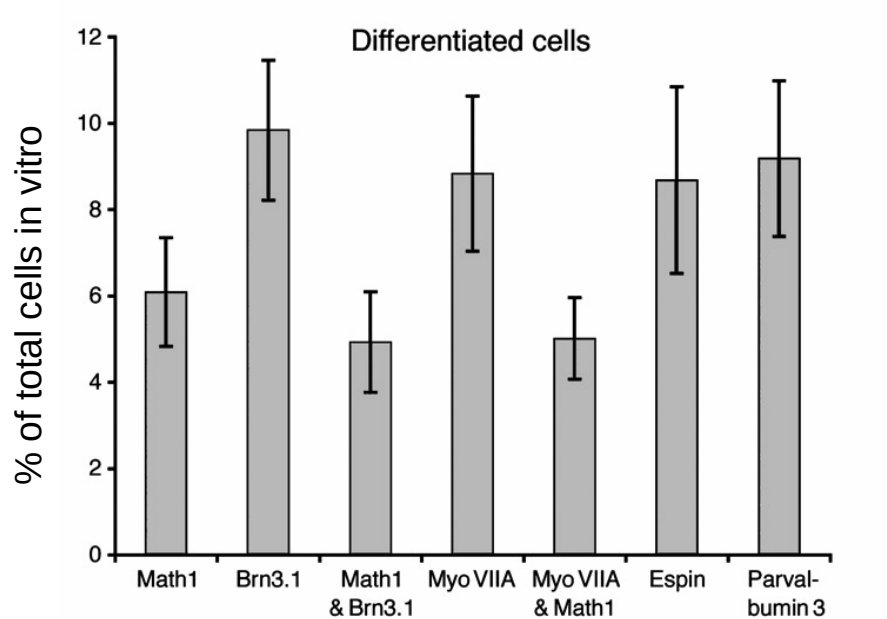

8 days
10 days
ES cells
Enriched progenitors
Differentiated cells
Otx2
Nestin
Pax2
BMP7
Jagged-1
p27Kip1
Math1
Myosin VIIA
Espin
Brn3.1
AchR α9
Gapdh
Early ear marker
Hair cell marker


Enriched progenitors
Nestin
B
DAPI Pax2
C
Differentiated cells
DAPI Math1
D
Myosin VIIA
E
Differentiated cells
Espin
F
Parvalbumin 3
G

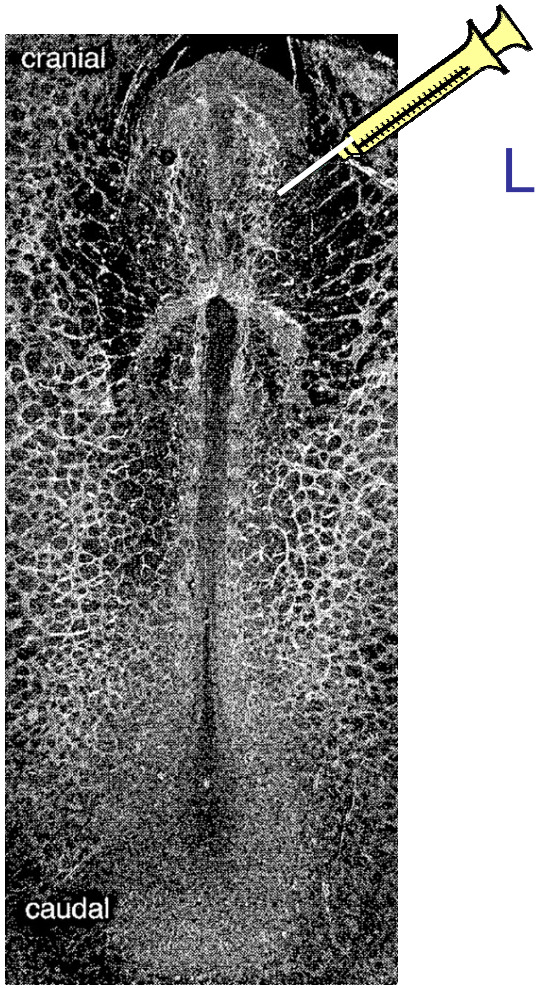
Hair cell markers are co-expressed.







Progenitor cells are grafted into chick embryos…

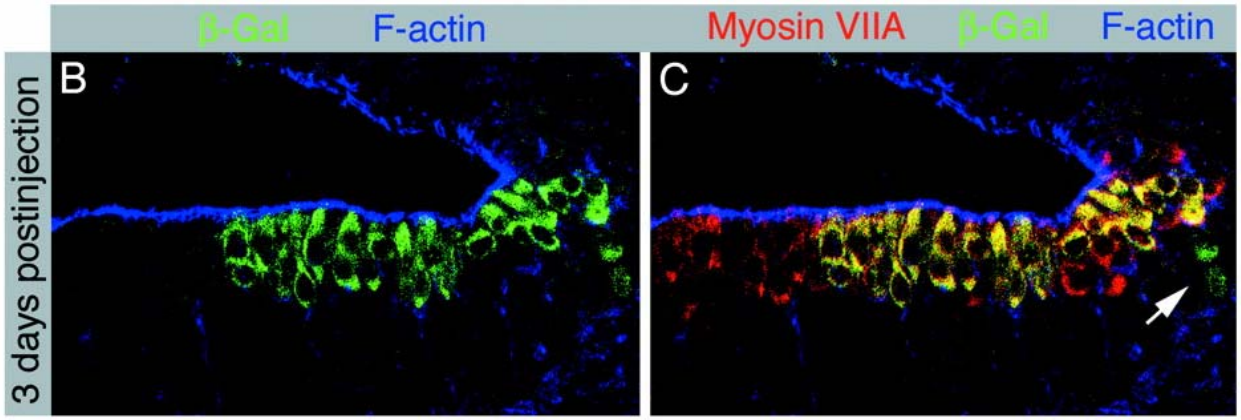


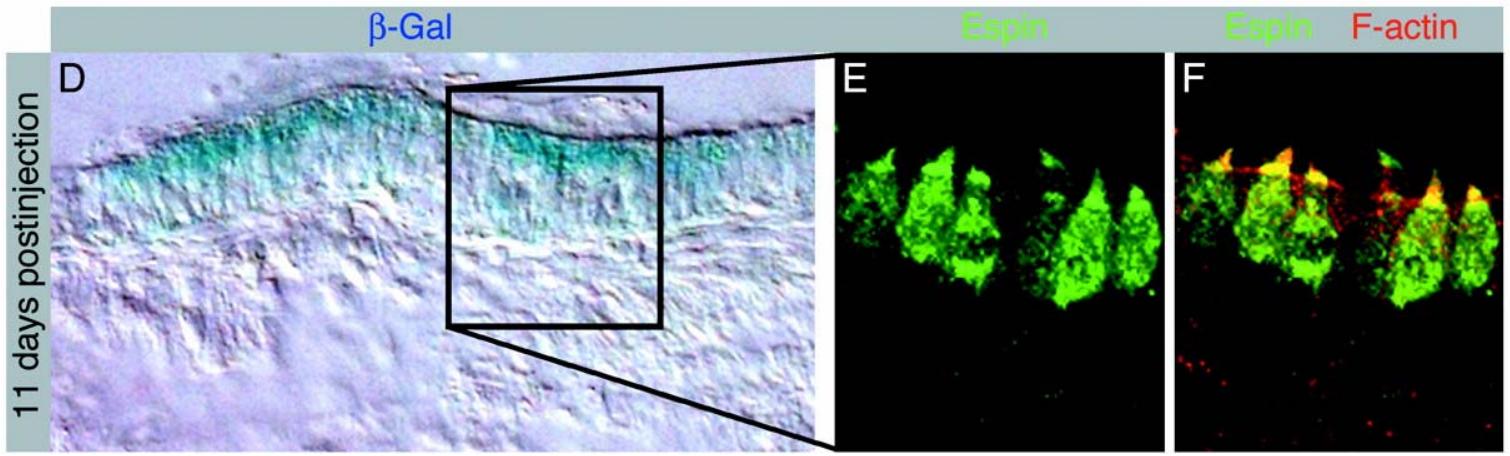
LacZ+ progenitor cells

Cells examined 3 & 11 days post-injection

Stage 17

Grafting promotes hair cell differentiation in the cochlea.





Implications

1. ES cells have the potential to differentiate into hair cells

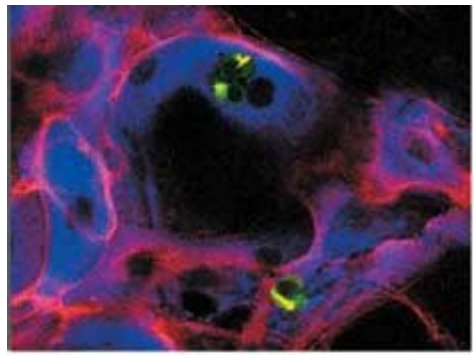
2. Auditory progenitor cells can be successfully created *in vitro*

3. Can this work in the adult cochlea?

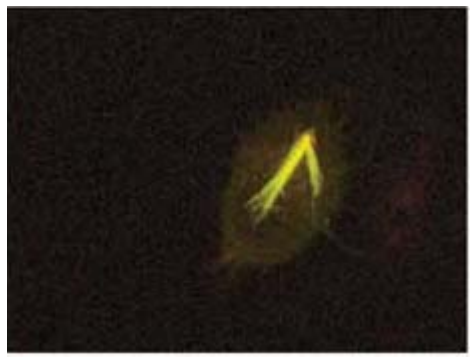
Adult Stem Cells in the Inner Ear

Utricle stem cells…

- Are highly proliferative
- Can self-renew *in vitro*
- Are pluripotent *in vivo*
- Can make hair cells *in vivo*



Myosin IIVA+ / Espin+
hair cells



Espin+ / f-actin+
stereocilia

Trans-differentiation in the Inner Ear

- Transcription factor *Math1* is required for hair cell differentiation
- *In vivo* transfection of cochlear support cells with *Math1* → trans-differentiation into hair cells
- These new hair cells attract growth of new auditory neurons!

Outer Ear Reconstruction

Performed since 1597 BC

Why repair: cosmetic reasons
improved hearing

Indications for reconstruction:

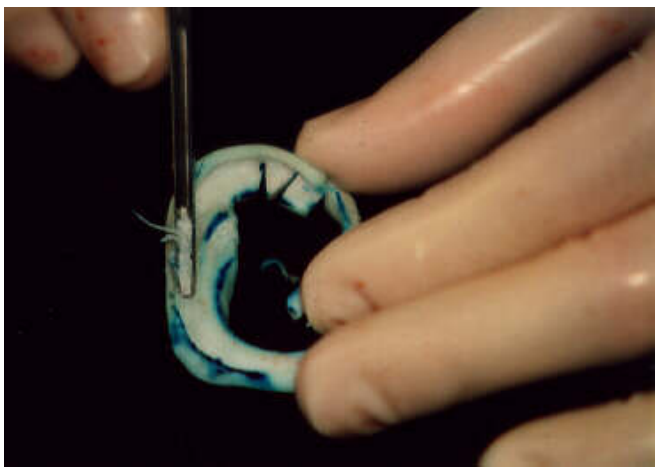
- Trauma
- Cancer removal
- Congenital abnormalities
  (1 per 7000 births)

Outer Ear Reconstruction

Gold standard: autologous rib cartilage

→ “unsatisfactory cosmetic results”

→ complications at secondary site



Shaping rib cartilage

Before

After

Tissue Engineering of Auricle

Aimed at creating a pre-molded, autologous cartilage auricle

Advantages

- Reduces/eliminates surgery at 2° site
- Provides more realistic auricle
- Less dependence on artistic skills of the prosthetist

Key Challenges

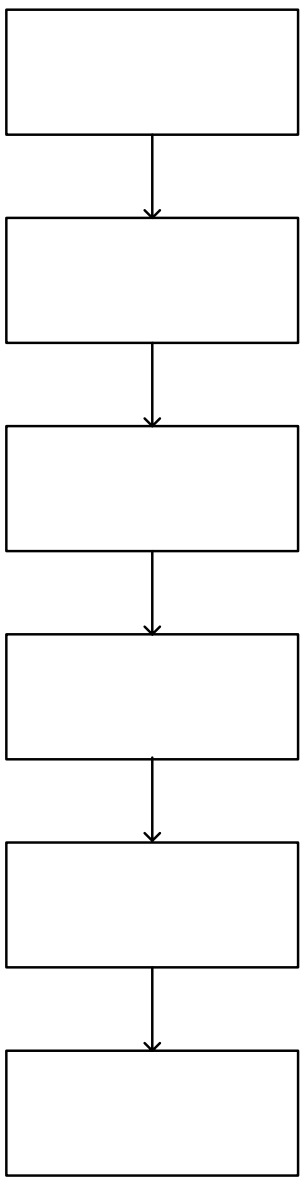
- Obtaining sufficient cartilage cells
- Creating appropriate anatomical shape
- Controlling polymer degradation in vivo
- Reducing immune reactivity

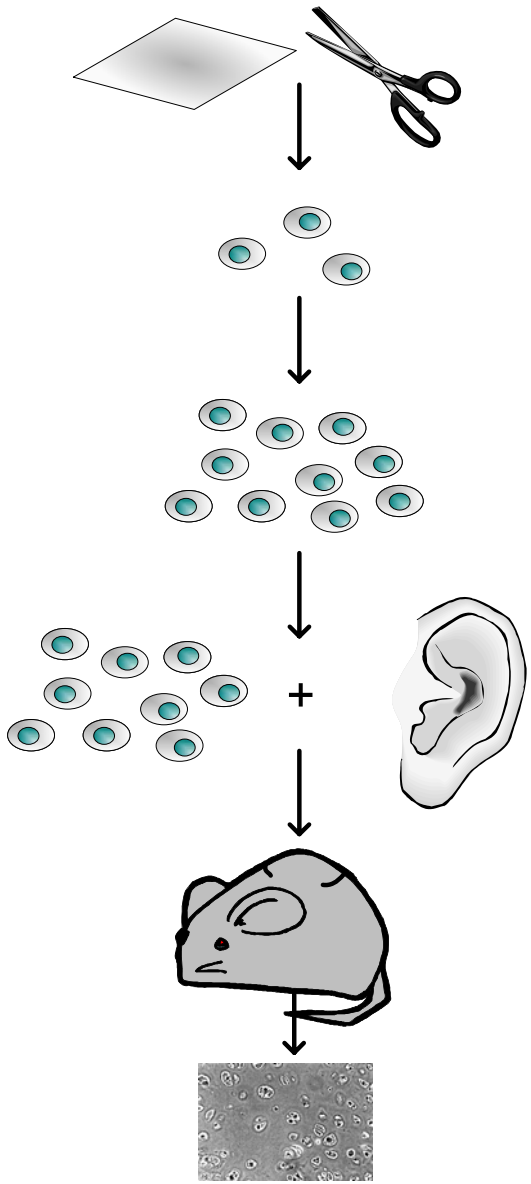
A tissue-engineering model for the manufacture of auricular-shaped cartilage implants

Haisch A, Klaring S, Groger A, Gebert C, Sittinger M

Key Challenges

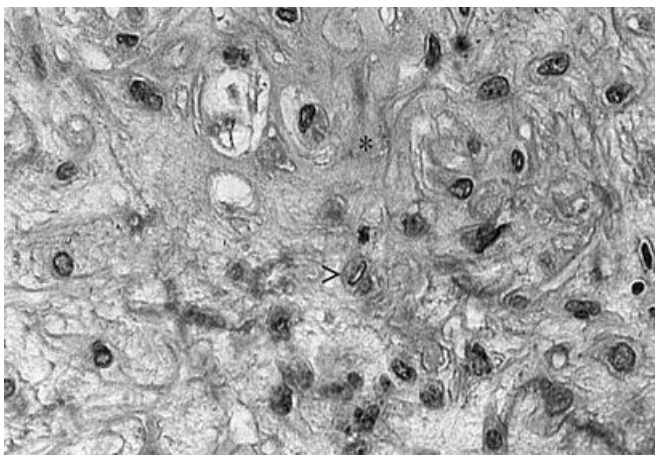
- ✓ Obtaining sufficient cells
  - Chondrocyte expansion in culture
- ✓ Creating appropriate shape
  - Pre-molding of PGLA scaffold
- ✓ Controlling polymer degradation
  - Co-polymer (PGLA and fibrin)
- ✓ Reducing immune reactivity
  - Use of autologous cells



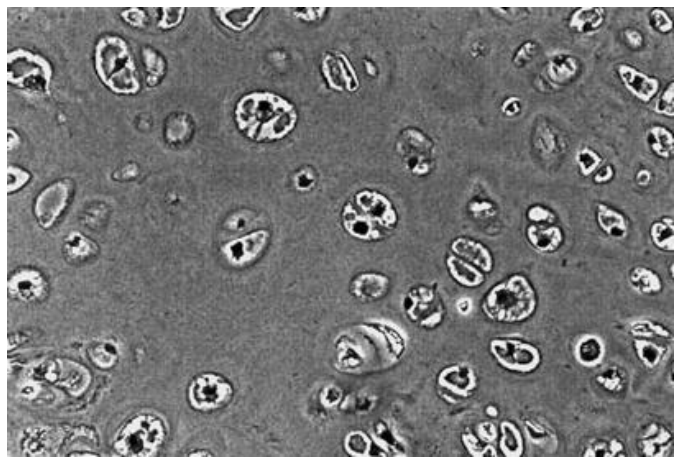


Results after 12 wks implantation…

- Smooth, whitish cartilage-like morphology
- Some collagen & proteoglycan present
- Encapsulated in fibrous tissue



6 weeks



12 weeks

Customized Ear Reconstruction?

- CT/MRI scans can be combined with rapid prototyping techniques to create custom reconstructions

- Research reduced to the university level

References

Li H,Roblin G, Liu H, Heller S. Generation of hair cells by stepwise differentiation of embryonic stem cells. *PNAS* 2003; 100:13495-13500

Li H, Liu H, Heller S. Pluripotent stem cells from the adult mouse inner ear. *Nature Medicine* 2003; 9(10):1293 - 1299

Kawamoto K, Ishimoto S, Minoda R, Brough DE, Raphael Y. Math1 gene transfer generates new cochlear hair cells in mature guinea pigs in vivo. *Journal Neuroscience* 2003; 23(11):4395-4400

Haisch A, Klaring S, Groger A, Gebert C, Sittinger M. A tissue-engineering model for the manufacture of auricular-shaped cartilage implants. *Eur Arch Otorhinolaryngol.* 2002; 259(6):316-321