OTOSTEM

HUMAN STEM CELL APPLICATIONS FOR
THE TREATMENT OF HEARING LOSS

PUBLISHABLE SUMMARY

FIRST PERIODIC REPORT

1 November 2013 – 30 April 2015

www.otostem.org

Co-funded by
the European Union

The lack of human otic cell models represents a significant roadblock hampering the development of drug-based or cell-based therapies. Hearing impairment is the most frequent human sensory deficit and is mainly caused by the irreversible loss of neurosensory cells in the cochlea. OTOSTEM addresses this urgent and unmet medical need for causal hearing loss therapies by focusing on human stem cell technology.

Concept

Our ability to hear and hence to communicate depends entirely on our auditory receptors – the sensory hair cells and their associated neurons that reside in the cochlear part of the inner ear. The exquisite sensitivity of the inner ear comes with the risk for damage for example by noise trauma, ototoxic drug damage, infections, age related degeneration and genetic causes. Once lost, the neurosensory cells of the ear are not replaced. This, in turn results in chronic hearing impairment, a devastating and highly prevalent disorder of infancy and adulthood with widespread implications for the individual and society as a whole. Adult hearing loss alone ranks among the five leading causes of burden of disease in Europe, entailing enormous socio-economic costs. Prosthetic treatment with hearing aids and cochlear implants is limited and reaches only every fifth patient. Due to the cause of the hearing loss – neurosensory cell loss – hearing aid amplification often fail to improve language comprehension and hence perform unsatisfactory.

Towards cell-based therapies (Fig. 1), OTOSTEM evaluates defined cell populations intended for therapeutic use for tumorigenic potential and validate their functional properties and biological potency in appropriate in vitro models.

Towards drug-based therapies (Fig. 1), cellular otic models are developed to the level of artificial sensory epithelia or "Mini Ear" in vitro models mimicking the in vivo organ equivalent.

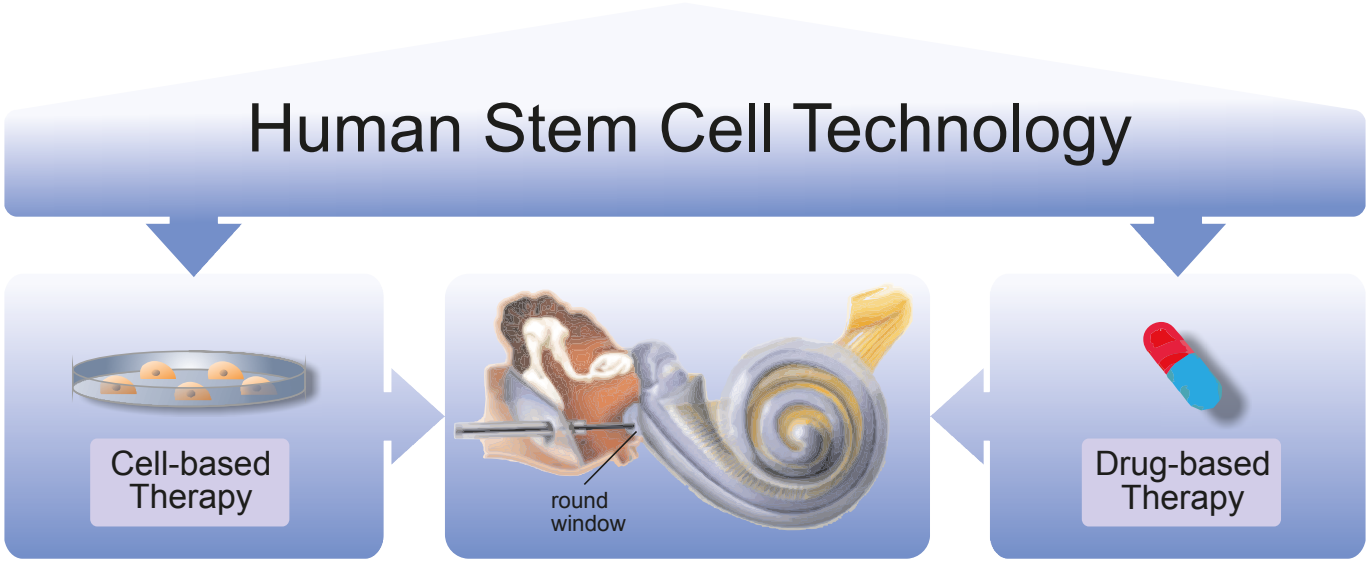

Figure 1: The two major applications for the therapeutic use of human stem cell technology for hearing loss are (i) direct cell based treatment by transplantation of human otic stem/progenitor cells into the cochlea and (ii) drug based treatment emanating from drug screening efforts for otoprotective and otoregenerative compounds. Both cells and drugs will be applied directly into the target organ – the cochlea –, which is self-contained and surgically accessible. An access route into the cochlear fluid space is provided through the round window membrane.

Otic cells have to be generated in sufficient numbers to allow screening in a high throughput/high content multi well plate format. In a subsequent step, these established and characterised cellular otic models are advanced into models of hearing loss. Exposure to ototoxic drugs allows for selective ablation of sensory hair cells and the establishment of "hearing loss in a dish" models for ototoxic drug screening.

One goal is to provide working assays for high throughput/content screens to the point that these assays can be used in the drug discovery setting represented by SMEs. Two therapeutic classes of drugs, one with otoprotective and the other one with otoregenerative effects are in the primary focus. Otoprotective effects aim to prevent cell death of human hair cells while otoregenerative effects aim at the replacement of lost human auditory neurons and hair cells.

Achievements in the first period

Controlling differentiation and proliferation in human otic stem cells

In improving the control over differentiation and proliferation in human otic stem cells from different sources (Fig. 2), this task provides a solid foundation for the whole project. The definition of protocols is based on functional and differentiation abilities at a single cell transcriptional level, and other important cell biological parameters.

The progress of this task included the development of a novel monolayer guidance protocol and validation of the existing protocols. This work was done in an effort to improve efficacy of human pluripotent stem cell guidance protocols towards the otic lineage.

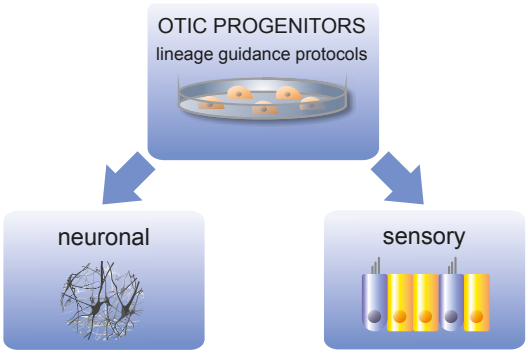

Figure 2: Pluripotent human cells from different sources are used as primary cell sources to generate purified otic progenitors using lineage guidance protocols and cell separation techniques. Purified otic progenitors are differentiated into neuronal and sensory epithelial cell types.

Otic progenitors cells suitable for cell transplantation

In this task, otic progenitor cells are investigated for their usage for transplantation. For cell transplantation purposes, enriched otic progenitors will be differentiated along sensory vs. neural lineages depending on the targeted tissue for cell replacement. Stem cell derived hair cells-like cells and stem cell derived neuron-like cells are assessed for their functional activity.

So far, Multi electrode array (MEA) setups, patch clamp and electric field assays have been established and are currently used to study several human models (Fig. 3).

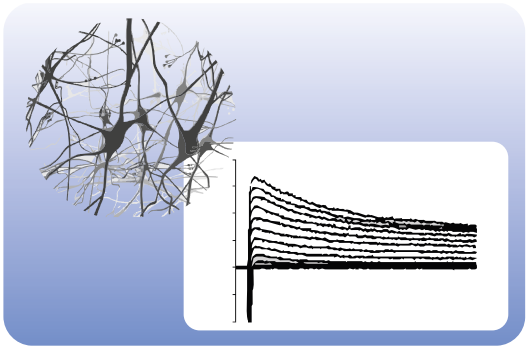

Figure 3: Purified otic progenitors and differentiated inner ear cell types will be tested for their function using electrophysiologic evaluation.

The OTOSTEM Consortium

Eberhard Karls Universität Tübingen (Coordinator)	DE
Universiät Bern	CH
The University of Sheffield	UK
Aix-Marseille Université	FR
Uppsala Universitet	SE
Stanford University	US
Massachusetts Eye and Ear Infirmary	US
Audion Therapeutics BV	NL
Acousia Therapeutics GmbH	DE
SCIPROM Sàrl	CH

Project Coordinator:

Prof. Dr. med. Hubert Löwenheim

info@otostem.org
www.otostem.org

The research leading to these results has received funding from the European Community's Seventh Framework Programme under grant agreement No. 603029.

Hearing loss in a dish model, ototoxicity, and drug screening

It is presumed that many drugs that are currently in use or in various stages of clinical trials have ototoxic potential. In this task, ototoxicity tests with human derived inner ear cells are established with aminoglycosides and cisplatin – known ototoxic drugs that cause hearing loss and are in widespread clinical use.

Cell based human otoprotection and otoregeneration bioassays do not exist. We are designing bioassays capable of identifying (a) otoprotective compounds that will prevent death of human hair cells, and (b) otoregenerative compounds that stimulate hair cell regeneration.

The consortium has now established several screening models and first compound screening efforts have been employed to identify ototoxic, otoprotective, or otoregenerative compounds (Fig. 4).

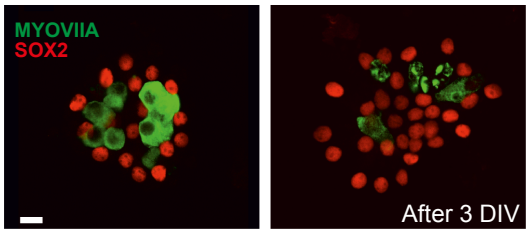

Figure 4: Mini Ear in control condition on the left with labelled supporting cells in red and hair cells in green. On the right is a Mini Ear that was exposed to an ototoxic compound (Neomycin) – labelling of hair cells in green is largely diminished.

Conclusion

In the first reporting period, the OTOSTEM project has progressed according to plan and several important issues were solved. However, to successfully apply human stem cells in the treatment of hearing loss, many more challenges have to be faced to prove the feasibility of the approach followed by the consortium.

The scientific and personal interactions have been inspiring and are promoting a good spirit throughout the consortium. Conserving and further developing this spirit will help the consortium to tackle the problems that lay ahead in a collaborative manner and lead to a successful completion of OTOSTEM.