Introduction
In humans, in whom speech and speech perception is essential for communication and social interaction, deafness is a severe disorder. One of the genetic non-syndromic forms is the autosomal recessive nonsyndromic deafness (DFNB) 9, which is caused by loss of function mutations in the OTOF gene encoding the protein otoferlin.

Established and characterized novel in vitro methods to transfer the full-length otoferlin into cochlear inner hair cells (IHC) in situ. Therefore, two different gene delivery methods, (i) electroporation (EP), as a cheap and flexible screening method with theoretically unlimited insert size and (ii) adenoviruses (Ad), which display high transduction efficiency, were evaluated.

Material and Methods
For the in vitro model system organs of Corti derived from C57Bl6 mice (wildtype (WT) and otoferlin-knockout (KO)) of embryonic day (E) 14.5 were cultured for up to 14 days. Using a square-wave electroporator, organ of Corti organotypic cultures were electroporated with the following settings: 27 V pulse amplitude, 25 ms pulse duration and 500 ms inter-pulse interval, 6-8 pulse trains. To transduce cultured organs of Corti, we used a second generation, replication-deficient adenovirus vector expressing eGFP and either otoferlinWT or otoferlin pga. To establish viral transduction in principle, a construct containing an eGFP sequence only was used.

Results
Our data show transfection efficiencies of E14.5 cultures in the range of ~2% of IHCs per organ of Corti on average using Electroporation. Transfection rates were dependent on (i) the pulse repetition rate, (ii) the construct composition and (iii) the age (rates dramatically decreased in older aged neonatal cultures (P0)).

Adenoviruses (Ad), displayed higher transduction efficiency, which depended on (i) age (transduction rates decreased in P0 cultures), (ii) time in culture prior to transduction and (iii) Ad-titer. In E14.5 cultures a titer of 3x10^6 transducing units (tu) produced significantly different transduction rates compared to 1x10^6 tu (11.83 ± 2.01% to 18.31 ± 3.50% (1x10^6 tu) vs. 27.61 ± 10.39% to 56.42 ± 7.53% (3x10^6 tu).

First pilot experiments with an Ad coding for otoferlin "pachanga" (pga) (harbours a point mutation that leads to decreased expression levels and functional impairment), I observed abundant transduction with otoferlinpga and eGFP in sensory hair cells. With the same construct cultures of Otoferlin-KO-mice were transduced, where otoferlinpga and eGFP signal could be detected. The same experiment was carried out with an Ad coding for otoferlinWT again transducing cultures of Otoferlin-KO-mice. Here, eGFP, but no otoferlin signal could be detected.

Discussion
In the present study, I implemented and characterized an organotypic culture system for the organ of Corti which showed signs of synaptic maturation and could be maintained for approximately 2 weeks. With Electroporation, transfection rates of hair cells varied a lot, the functionality of Electroporation for studies of gene expression in hair cells seems limited to proof-of-principle approaches. After establishing viral transduction, I went on to transduce otoferlin-KO cultures to either express the mutated otoferlinpga or WT otoferlin. Here, eGFP, but no otoferlin signal could be detected.