00697

THEME : INNOVATIVE TECHNOLOGIES AND TRANSLATIONAL THERAPIES

Microneedle-mediated delivery of AAV through the round window membrane for inner ear gene therapy

Eugénie Breil, Ghizlène Lahlou, François Voruz, Daniella Hébert, Sharon J. Feng, Michelle Yu, Aykut Aksit, Elizabeth S. Olson, Marie-José Lecomte, Saaid. Safieddine, Jeffrey W. Kysar, Anil K. Lalwani

Introduction

- Intra-cochlear gene therapy, especially with adeno-associated virus (AAV), is a promising solution for correcting genetic hearing loss.
- An innovative 100µm-mironeedle designed to significantly reduce potential trauma to the round window membrane (RWM) has been developed in our laboratory[2].
- Tested in guinea pigs[3] and human temporal bone[4], this technique separates fibers without tearing them. Complete healing occurs in 48h with no hearing loss[5].

The use of a microneedle for the administration of viral vectors could be a minimally invasive alternative for intra-cochlear gene therapy.

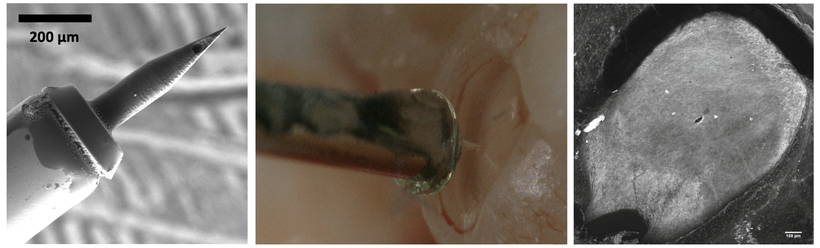

Fig.1: A.Scanning electron microscopy of 3D-printed hollow microneedle fabricated via 2-photon polymerization lithography. B. Digital microscopic image of a microneedle in contact with a harvested guinea pig RWM C. Confocal image of the entire guinea pig RWM with a perforation made by the microneedle.

Results

For all three vectors with no significant difference :

- Successful hair cells transduction within all cochlear turns
- On average half of all inner hair cells transduced
- Decreasing gradient from base to apex

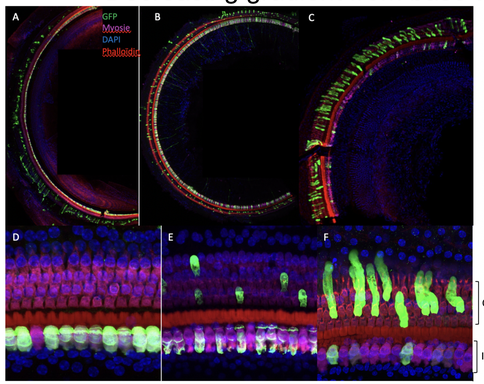

Fig2: transduction profile of the cochlea after microneedle-injection of AAV2-CAG-GFP. Immunostaining by GFP (green), myosinVIIa (magenta), phalloidin (red) and DAPI (blue). Base (A/D), middle turn (B/E) and apex (C/F) of the cochlea at x20/x40 magnification observed by confocal microscopy.

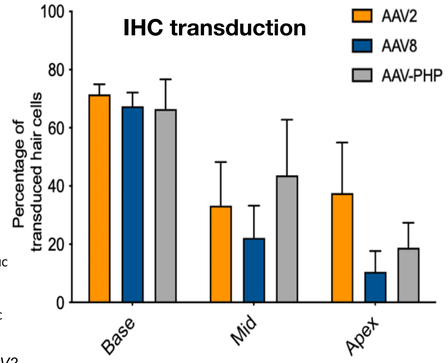

Fig3: percentage of cell transduction for the 3 AAV serotypes, according to the cochlear region (base, middle turn and apex) for inner hair cells (IHC)

No significant hearing loss before perforation, immediately after perforation and at one month follow-up.

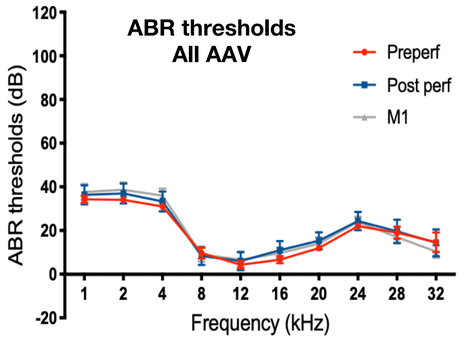

Fig4: Average ABR thresholds and SD of right ears after injection with 3 different serotypes of AAV (n=15) before perforation of the RWM (red curve), immediately after perforation (blue curve) and at 1 month (grey curve).

Aims

To study tropism and tolerance of microneedle-mediated intracochlear injection of AAV through the round window membrane in guinea pigs.

Conclusion

The microneedle is a safe, reliable, and effective tool for AAV administration in guinea pigs, enabling successful hair cells transduction and preserved hearing thresholds at one month after perforation of the RWM, paving the way for atraumatic cochlear gene therapy.

Methods

- An intracochlear delivery of 5µL of 3 vectors (AAV2 (n=5), AAV8 (n=5), AAV-PHP (n=5), and artificial perilymph (n=5) with the 100µm-diameter hollow microneedle at a rate of 1 µL/min, through the RWM of young guinea pigs (n=20).
- Distortion product otoacoustic emissions (DPOAE) and auditory brainstem response (ABR) were performed prior to, immediately after injection, and at one month follow-up.
- Cochleae were fixed and decalcified for hair cell analysis with confocal microscopy.

References

1 1. Qi, J., F. Tan, L. Zhang, et al., *AAV-Mediated Gene Therapy Restores Hearing in Patients with DFNB9 Deafness.*Advanced Science, 2024. **11**(11): p. 2306788. DOI: 10.1002/advs.202306788. *2. Aksit A, Rastogi S, Nadal ML, et al. Drug Delivery Device for the Inner Ear: Ultra-sharp Fully Metallic Microneedles. Drug Deliv Transl Res. 2021;11(1):214-226. doi:10.1007/s13346-020-00782-9*

3. Aksit A, Arteaga DN, Arriaga M, et al. In-vitro Perforation of the Round Window Membrane via Direct 3-D Printed Microneedles. Biomed Microdevices. 2018;20(2):47. doi:10.1007/s10544-018-0287-3

4. Chiang H, Yu M, Aksit A, et al. 3D-printed Microneedles Create Precise Perforations in Human Round Window Membrane in situ. Otol Neurotol Off Publ Am Otol Soc Am Neurotol Soc Eur Acad Otol Neurotol. 2020;41(2):277-284. doi:10.1097/MAO.0000000000002480

5. Yu M, Arteaga DN, Aksit A, et al. Anatomical and Functional Consequences of Microneedle Perforation of Round Window Membrane. Otol Neurotol Off Publ Am Otol Soc Am Neurotol Soc Eur Acad Otol Neurotol. 2020;41(2):e280-e287. doi:10.1097/MAO.0000000000002491

Institut de l'Audition | INSTITUT PASTEUR | COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK

WCA World Congress of Audiology 19-22 September 2024 Paris, France CNIT Paris La Défense