Anatomic, Physiologic, and Proteomic Consequences of Repeated Microneedle-Mediated Perforations of the Round Window Membrane

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Stephen Leong, BA, Aykut Aksit, MS, Betsy Szeto, MD, MPH, Xiang Ji, Rajesh K. Soni, PhD,
Elizabeth S. Olson, PhD, Jeffrey W. Kysar, PhD, Anil K. Lalwani, MD
Department of Otolaryngology—Head & Neck Surgery, Columbia University Irving Medical Center, New York, NY
Department of Mechanical Engineering, Columbia University, New York, NY

Abstract

Background: We have developed 3D-printed microneedle technology for diagnostic aspiration of perilymph and intracochlear delivery of therapeutic agents. In this study, we investigate the anatomic, physiologic, and proteomic consequences of microneedle-mediated perforations of the same round window membrane (RWM) at multiple timepoints.
Methods: Hollow microneedles were introduced into the tympanic bulla of Hartley guinea pigs (n=8) and the RWM was perforated; 1 μL of perilymph was aspirated from the cochlea. 72 hours later, an additional 1 μL of perilymph was aspirated. Pre- and post-intervention hearing tests (compound action potential (CAP) and distortion product otoacoustic emissions (DPOAE)), confocal microscopy, and perilymph proteomic analysis were conducted for 7/8 animals.
Results: Hearing tests demonstrated mild hearing loss at 1-4 kHz most consistent with conductive hearing loss. Confocal microscopy demonstrated complete healing of all perforations with full reconstitution of the RWM. Perilymph proteomic analysis identified 1855 proteins across 14 samples (2 aspirations each for 7 animals). Paired t-tests with $p < 0.01$ revealed significant changes in 13 of 1855 identified proteins (0.7%) between the first and second aspirations.
Conclusions: We demonstrate that repeated microneedle perforation of the RWM is feasible, does not directly cause hearing loss, allows for complete healing of the RWM, and does not significantly change the proteomic expression profile. Thus, microneedle-mediated repeated aspirations in a single animal can be used to monitor the response to treatments over time.

Introduction

- Single microneedle-mediated RWM perforation does not cause hearing loss, heals within 48-72 hours, and yields sufficient perilymph for proteomic analysis[1,2,3,4].
- The advent of intracochlear gene therapies necessitates a means for extended treatment monitoring to ensure continued efficacy and safety.

Methods and Materials

- 100-μm-diameter hollow microneedles were synthesized using 2PP lithography.
- The tympanic bullae of Hartley guinea pigs (n=8) were opened with adequate exposure of the RWM; DPOAE and CAP were recorded to assess hearing pre- and post-intervention.
- Hollow microneedles were introduced into the bulla and the RWM was perforated; 1 μL of perilymph was aspirated from the cochlea over the course of 45 seconds. 72 hours later, an additional 1 μL of perilymph was aspirated.
- RWMs were harvested 72 hours following the second aspiration for confocal imaging.
- Perilymph proteomic analysis was completed using mass spectrometry-liquid chromatography.
- Two-tailed paired t-tests and repeated measures ANOVA tests were used to evaluate for significance at $p < 0.01$.

Results

Figure 1. Scanning electron microscope (SEM) image of a hollow microneedle (100-μm outer diameter, 35-μm inner diameter).

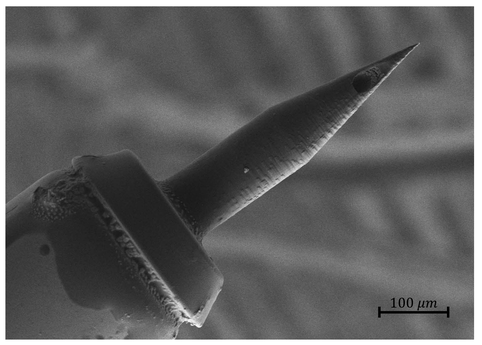


Figure 2. CAP measurements taken prior to the first perforation (survival 1, blue) and second perforation (survival 2, red), and 72 hours following the second perforation (nonsurvival, green). 95% confidence intervals are bound by the shaded areas.

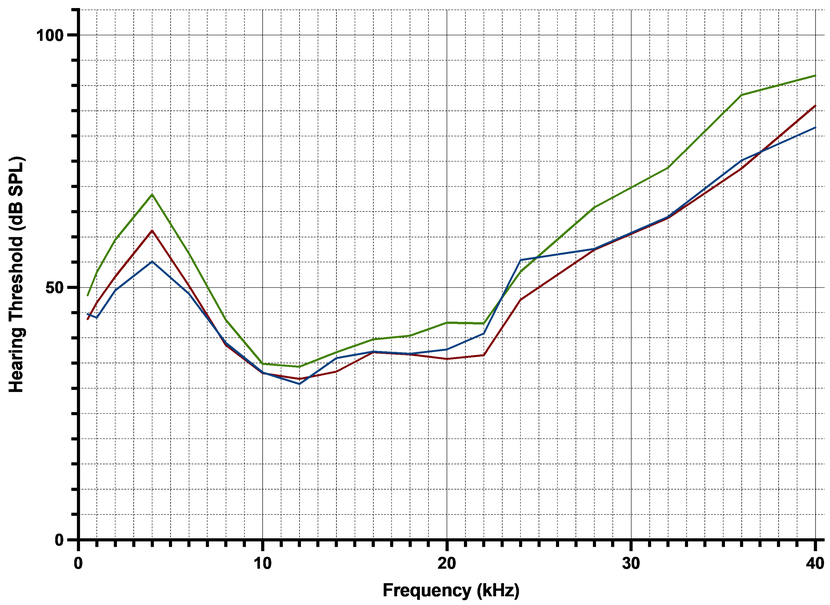


Figure 3. DPOAE measurements taken before and after the first perforation (left) and second perforation (right). Blue lines correspond to pre-perforation DPOAE and red lines correspond to post-perforation DPOAE. 95% confidence intervals are bound by the shaded areas.

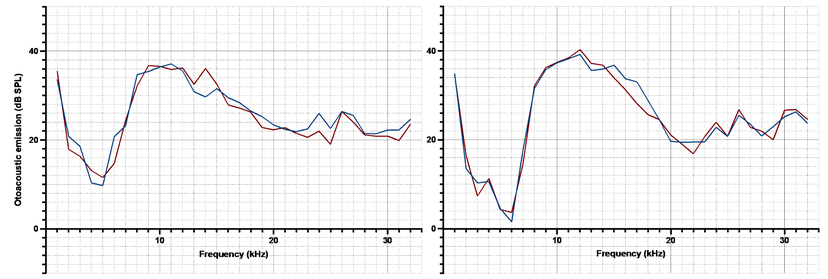


Figure 4. Functional location of proteins with significant changes between the first and second perforations.

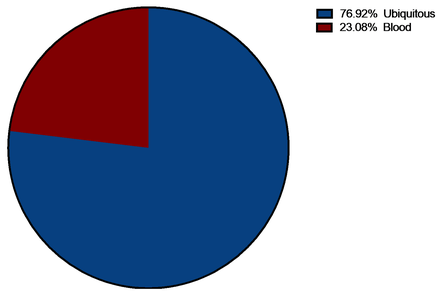


Figure 5. Proteins with significant changes between the first and second perforations. Fold changes are displayed, with red bars indicating a decrease in intensity and blue bars indicating an increase in intensity.

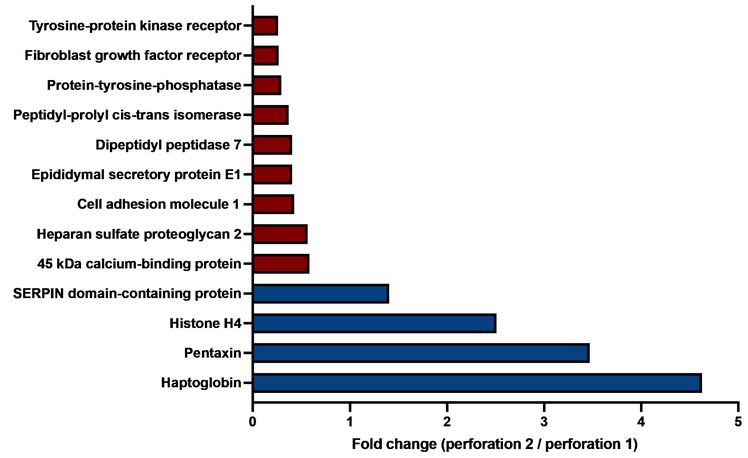
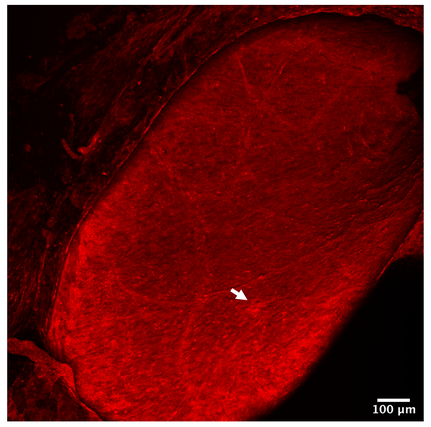


Figure 6. Confocal image of a healed RWM after two perforations. The arrow indicates healing tissue following the second perforation; the first perforation cannot be visualized.



Discussion

- Mild hearing loss at 1-4 kHz most likely represents conductive hearing loss; during the final surgery, extensive middle ear effusion and debris were noted, likely due to repeated access to the middle ear over a short timeframe.
- Full reconstitution of the RWM after two perforations, and comparable DPOAEs before and after each perforation, demonstrates that repeated perforations are safe.
- The inner ear protein cochlin was identified in all samples.
- Only 0.7% of proteins had a significant change between the first and second perforations, which is below the accepted type I error rate of 1%.
- Given that the identified proteins do not have inner ear or inflammatory functions, the significant changes are more likely to be the result of type I error.

Conclusions

- We demonstrate that repeated microneedle perforation of the RWM is feasible, does not directly cause hearing loss, allows for complete healing of the RWM, and does not significantly change the proteomic expression profile.
- Microneedle-mediated repeated aspirations in a single animal can be used to monitor the response to inner ear treatments, such as gene therapy, over time.

Contact:
Stephen Leong, BA
Columbia University Vagelos College of Physicians & Surgeons
sl3793@cumc.columbia.edu

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