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From Bench to Booth: Examining Hair-Cell Regeneration Through an Audiologist's Scope

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Abstract

Damage to auditory hair-cells is a key feature of sensorineural hearing loss due to aging, noise exposure, or ototoxic drugs. Though hair-cell loss is permanent in humans, research in bird species led to the discovery that analogous hair-cells of the avian basilar papilla are able to regenerate after being damaged by ototoxic agents. Regeneration appears to occur through a combination of the mitotic expansion of a precursor population of supporting cells and direct transdifferentiation of supporting cells into functioning hair-cells. This review will synthesize the relevant anatomy and pathophysiology of sensorineural hearing loss, the historical observations that led to the genesis of the hair-cell regeneration field, and perspectives on initial human hair-cell regeneration trials.

Keywords
- hearing
- auditory
- cochlear implants

For many years, audiology patients with gradual onset sensorineural hearing loss have been informed that their hearing loss is permanent due to irreversible damage to their cochlear hair-cells, which are essential sensory structures of the inner ear.[1–3] To help compensate for this permanent sensory deficit, patients are offered amplification technologies such as hearing aids. While hearing aids can amplify sounds such as speech and other informative environmental stimuli, they do not restore the impaired physiology of the inner ear. As a result, patients with sensorineural hearing loss who use hearing aids often report continued difficulty understanding speech, particularly in noisy environments where current amplification processing strategies fail to reduce ambient noise to an extent that optimizes speech processing.[4]

To restore normal hearing function to patients with significant sensorineural hearing loss, the root cause of the hearing loss must be addressed directly. In most cases, the primary site of lesion is the auditory hair-cell and nearby structures.[5] Interestingly, although hair-cell damage is permanent in mammalian cochleae, nonmammalian vertebrates such as birds regenerate hair-cells and thereby restore hearing function.[6–10] This review focuses on the historical perspectives of the discovery of hair-cell regeneration and the field of study that originated from this significant finding.

Microanatomy of the Inner Ear: Pathophysiology of Sensorineural Hearing Loss

The hearing organ of the inner ear, the cochlea, is a fluid-filled coiled structure (►**Supplementary Fig. S1A**) that contains the sensory epithelium called the organ of Corti (►**Supplementary Fig. S1B, C**). The microanatomy of a normally functioning cochlea differs from that of patients with overt sensorineural hearing loss due to changes in many structures, but most notably hair-cells.[3] Normal sensory epithelium contains neatly arranged rows of hair-cells along the length of the cochlea, with precisely organized stereocilia sitting atop each hair-cell body (►**Supplementary Fig. S1D**). The arrangement of these stereocilia in a consistent direction

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ensures the population of hair-cells has consistent mechanosensory transduction throughout the auditory organ as the cells convert the small mechanical movements of the stereocilia into a series of downstream signals, thereby propagating the information further along the auditory pathway.[11]

Stereocilia trigger a series of distinct processes in inner and outer hair-cells, which act together to support proper representation of sound by the inner ear and transmission of that information to the auditory pathways in the brain. The motility of the outer hair-cell allows for precise magnification of the local signal to the inner hair-cell, which supports greater frequency tuning and dynamic range compression.[12–14] Meanwhile, the inner hair-cell is responsible for relaying this amplified and tuned signal to the ascending auditory pathway.[15]

Aging, noise exposure, ototoxic medications, and a variety of genetic and acquired conditions can all lead to hair-cell damage. Damage to mammalian hair-cells results in reorganization of the auditory epithelium to remove the hair-cell from the surface of the epithelium.[16] Severe damage results in a dead region lacking any sensory structures[17]; in some cases, the auditory epithelium could be reduced to a relatively flat dedifferentiated structure devoid of hair-cells or any differentiated supporting cells.[18,19]

Noise exposure affects many aspects of the sensory epithelium, from the tips of the stereocilia[20,21] to the supporting cells that sit atop the basilar membrane. As noise exposure does not selectively or uniformly affect the auditory epithelium, many studies of hair-cell regeneration employ ototoxic agents to improve the specificity and consistency of their findings.[22–25]

Seminal Discoveries in Hair-Cell Regeneration and Development of Avian Models

Efforts to study hair-cell development, damage, and death were consistently underway by the 1980s. During that period, embryologic development in model organisms was utilized to characterize the gross morphological changes that occur during maturation, as well as the development of the tonotopic organization of the auditory epithelium.[26–30]

Model organisms offered certain advantages over studying human physiology directly while taking advantage of some traits that are conserved between species, such as the aforementioned tonotopic organization of the auditory epithelium. This tonotopy is conserved in the avian basilar papilla, a structure that is generally flat (►**Supplementary Fig. S2A**) when compared with the spiraled mammalian cochlea (►**Supplementary Fig. S1A**), allowing researchers to more quickly process and image their findings. However, there are notable differences in the overall organization of the avian auditory epithelium, such as the regular interdigitation of generally undifferentiated supporting cells between each hair-cell (►**Supplementary Fig. S2B**), as well as the distribution of hair-cells across the entire epithelium, appearing as a field of hair-cells (►**Supplementary Fig. S2C**) instead of the distinct rows of hair-cells found in the mammalian organ of Corti (►**Supplementary Fig. S1C, D**). Despite these differences, the causes and apparent mechanisms of hair-cell damage and death in the mammalian cochlea are considered comparable to that of the avian basilar papilla, and therefore, the basilar papilla was the preferred choice for studies of frequency specificity of noise damage and the location of damage along the length of the epithelium.

Similar to the mammalian organ of Corti, the avian basilar papilla has two populations of hair-cells: short hair-cells and tall hair-cells (►**Supplementary Fig. S2B**). Tall hair-cells are similar to the inner hair-cells, while short hair-cells are considered to be most like the outer hair-cell population of the organ of Corti.[31] Outer hair-cells are well known to be responsible for the active mechanism frequently referred to as the cochlear amplifier, due to somatic electromotility, in which the cell body shortens its length to increase frequency selectivity.[14] Meanwhile, short hair-cells of the avian basilar papilla appear to exhibit both active hair bundle movements along with somatic electromotility[32,33] to start approaching this goal. Similar to outer hair-cells, which are more sensitive to damage than inner hair-cells, short hair-cells also exhibit greater sensitivity to damage relative to their tall counterparts.[34] Patterns of damage can vary depending on the type of treatment applied to the organ, with differential susceptibility across mammalian and avian species.

Avian Hair-Cells Exhibit a Unique Ability to Regenerate

While studying the process of hair-cell damage to the auditory epithelium of the chicken, researchers discovered a totally unexpected phenomenon. A few weeks after auditory hair-cells were damaged in the mature avian hearing organ, several healthy-looking cells began to appear in the region of damage. On closer examination, these cells appeared consistent with newly developing hair-cells.[35,36] Subsequent studies confirmed that these were indeed new hair-cells which had regenerated after the original hair-cells were damaged.[37,38]

This groundbreaking finding would challenge a decades-old assumption in auditory neuroscience, calling into doubt the dogma that hair-cells cannot differentiate de novo after damage to a warm-blooded vertebrate's cochlea, and supporting the idea that regeneration of hair-cells could be possible under the proper conditions. This spurred an entirely new field of investigation into the regeneration of hair-cells in the nonmammalian inner ear, in the hope that one day researchers could translate those findings to the mammalian ear to treat sensorineural hearing loss.

Avian Hair-Cells Regain Function after Damage, Restoring Auditory Sensitivity after Regeneration

The finding that hair-cells regenerate in adult avian species led to many follow-up studies on the consequences of hair-cell damage and subsequent regeneration as assessed through electrophysiological and behavioral measures. As in mammals, the avian basilar papilla produces otoacoustic emissions (OAEs) and auditory brainstem responses (ABRs).

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OAEs are often lower in baseline magnitude in nonmammalian vertebrates when compared with normal hearing mammals,[39] which may be a consequence of the underlying electromotility differences between outer hair-cells of the mammal and short hair-cells of the nonmammal. Despite these differences, OAEs are lost after hair-cell damage and generally restored after hair-cells regenerate,[40] following the trend of other measures.

ABRs assess the functional status of the ear and the auditory neural pathway by referencing well-defined properties of the ABR for a given species. As these objective measures require no behavioral training, they are often used in animal studies to assess outcomes of hair-cell damage and regeneration in the avian basilar papilla. Therefore, ABRs are particularly useful when examining hearing threshold changes and growth functions throughout the period of hair-cell damage and subsequent hair-cell regeneration, and a large number of studies have shown thresholds worsen after damage and are restored to normal or near-normal levels after regeneration occurs.[9,10,41,42] While these objective measures can be used to verify hair-cell function similar to audiology patient populations (particularly for pediatric visits), the presence of OAEs and ABRs may not guarantee normal hearing for an individual as it does not capture central auditory perception. Behavioral measures are required to better assess how an individual's hearing truly functions.

Behavioral studies of animals are challenging due to the extensive training needed to ensure the animal will be able to reliably complete a task. Despite these challenges, early pioneers of hair-cell regeneration studies trained animals to complete behavioral protocols to better understand how hair-cell regeneration affects hearing perception beyond that which is measured by standard histologic and electrophysiologic methods. The budgerigar, a small Australian parrot, was investigated as a model system due to the ability to test the perceptual consequences of hair-cell damage and regeneration by analyzing the bird's ability to respond to natural or synthetic contact calls. When hair-cells are damaged, auditory thresholds are elevated and the ability to accurately classify bird calls is impaired,[7] which is similar to the clinical finding that elevated thresholds may be found in a patient with sensorineural hearing loss and an inability to accurately recognize words or phonemes. Vocal production can also be affected when birds are deafened, as the Bengalese finch demonstrates a degradation in song quality after deafening because auditory feedback is required for maintenance of the stable adult song.[43]

While thresholds are restored 8 weeks after damage, when hair-cell regeneration is well underway, the ability to classify bird calls requires extended time after hair-cell regeneration to be restored to normal.[7] Similarly, the Bengalese finch follows a similar time course to regenerate hair-cells, with song quality significantly improved but not completely restored to normal by 8 weeks after damage.[43] These results indicate that, although auditory thresholds are essentially restored 8 weeks after many hair-cells were regenerated, more time is necessary for central processing to return and for normal hearing perception and associated behaviors to be restored in the avian model. If the same process is required for audiology patients who undergo hair-cell regeneration trials, more time will be needed after hair-cells regenerate to allow for central auditory processing to be fully restored to normal.

Hair-Cell Regeneration Improves Pure-Tone Thresholds in Animals

An important initial area of research was the effect of hair-cell regeneration on pure-tone thresholds, the standard approach to diagnose hearing loss for audiology patients. Investigators trained European starlings to respond to pure tones, and they were able to obtain thresholds from these birds on a daily basis after exposing them to ototoxic drugs that led to hair-cell loss.[44] Pure-tone thresholds began to recover soon after ototoxic drug administration was ceased and continued to improve over the course of 50 days. Despite the dramatic recovery in pure-tone thresholds, they did not improve completely to baseline levels, with some thresholds permanently shifted by 5 to 15 dB SPL between 4 and 6 kHz and a 25 dB shift at 7 kHz (the highest trained frequency). The stereocilia of the hair-cells in the high-frequency region were misoriented, which may have contributed to this maintained shift in pure-tone thresholds. Interestingly, subsequent repeated administration of aminoglycosides led to a lesser degree of additional threshold shifts,[44] which suggests that regenerated hair-cells could be less susceptible to ototoxic effects compared with the original hair-cells.

Although there is some degree of permanent hearing threshold shift after damage to the auditory epithelium, hearing restoration occurs prior to full maturation of the hair-cells, starting as early as 1 week after damage. Most recovery in pure-tone thresholds is observed by 4 weeks after damage, although hair-cells continue to regenerate for up to 8 weeks after damage.[10] Despite any lingering threshold shifts after damage, temporal and spectral perception, along with the ability to recognize and produce complex bird calls, appears to demonstrate a return to normal after severe damage.[8,43,45,46]

As the naturally regenerative basilar papilla appears to have some permanent damage at higher frequencies or when extensive damage is sustained to supporting cell populations, these limitations are important to consider as we move toward mammalian hair-cell regeneration studies. The vast majority of sensorineural hearing impairment due to noise exposure or age-related hearing loss in humans is in the high-frequency range, so this finding[44] could explain ongoing difficulty with regenerating structures in the high-frequency ranges, which are critical to restore for audiology patients who require better access to high-frequency speech sounds. Despite these limitations, the ability to significantly improve hearing for those with severe-to-profound hearing loss offers potentially improved outcomes when compared with that of traditional hearing aids or cochlear implants.

Uncovering Cellular Mechanisms that can Drive Hair-Cell Regeneration

Initial efforts to determine the mechanism by which hair-cells were regenerating in the avian model required the identification of the precursor cell population. The pioneers

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in this field used a molecular tracing tool to label cells undergoing mitotic division in the area near the hair-cells after damage. They found supporting cells and hair-cells both incorporated this tracing label during the regenerative phase after damage, which suggested the supporting cells represent a progenitor cell population from which newly generated hair-cells are derived.[37,38] This major breakthrough demonstrated that the supporting cells could be targeted as a local native cell population from which replacement hair-cells develop. Further, it demonstrated that supporting cells undergo mitotic division (►**Supplementary Fig. S3C**), which allows for replenishment of the supporting cell population as well as regeneration of newly differentiated hair-cells. Replenishment of the supporting cell population along with hair-cells could help ensure the auditory epithelium retains the proper mass and stiffness to restore frequency selectivity along the length of the auditory epithelium.

Later studies[47–49] would go on to demonstrate that supporting cells can also directly change their morphology and function into that of a hair-cell, a process referred to as direct transdifferentiation (►**Supplementary Fig. S3D**). This process begins shortly after hair-cell damage and results in the production of newly differentiated hair-cells more quickly than hair-cells produced by mitotic division. In addition, predominant mechanisms of hair-cell regeneration differ along the width of the basilar papilla; mitotic division occurs more often in the neural region, whereas direct transdifferentiation is more common in the abneural region.[47]

Just as short and tall hair-cells are regenerated in the avian basilar papilla, regenerative therapy aims to yield both outer and inner hair-cells in the proper regions of the regenerated organ of Corti. However, the characteristic differences between the basilar papilla and the organ of Corti may present a challenge to the process. Regenerating outer and inner hair-cells will not only require precise placement of these cell populations but also the restoration of the active mechanism from the outer hair-cell population to yield greater sensitivity than the inner hair-cells alone provide. The active mechanism requires a complex series of cellular and subcellular behaviors that are precisely timed in certain areas of the cochlea, which relies on restoration of stereocilia in the proper alignment as well as proper balance of mass and stiffness of the basilar membrane, which is in turn affected by the number of cells present in a certain region of the cochlea.

After the supporting cell population was identified as the source of newly developing hair-cells, investigators turned their attention to the molecular mechanisms controlling these and other progenitor cells, such as stem cells, in hopes that they could induce cells to differentiate into hair-cells in the mammalian cochlea. While other articles in this issue discuss more recent research related to the field of mammalian hair-cell regeneration, understanding the early successes and challenges in this field of research can be useful to better understand potential treatment trajectories as we move to human trials of hair-cell regeneration. A brief introduction to early investigations of mammalian hair-cell regeneration is included here as a bridge toward understanding initial outcomes of human trials.

Bridging from Avian to Mammalian Hair-Cell Regeneration

To begin studying mammalian hair-cell regeneration, auditory neuroscientists first sought to understand how hair-cells differentiate in newly developing mammalian auditory epithelium. Traditionally, scientists manipulate an intrinsic property of the tissue (e.g., through changing gene expression patterns) or an extrinsic property of the tissue (e.g., through changing the molecular environment) to evaluate outcomes at a cellular level. For example, researchers can introduce a treatment that forces expression of a particular gene and/or increases the presence of a certain environmental molecule. After the treatment, the tissue is observed to determine how that particular change affected the fate of a cell (e.g., the transition from supporting cell to hair-cell).

While humans develop auditory hair-cells in utero and are born equipped with a fully functioning set of hair-cells in each cochlea, mice continue to develop and differentiate their auditory epithelia immediately after birth during their postnatal period. For in vivo studies (e.g., manipulating gene expression within a live animal), this postnatal period of the mouse provides improved access for researchers to evaluate cellular outcomes of the auditory epithelium as the mouse completes the final stages of hair-cell differentiation. This postnatal development period also allows for in vitro evaluation of the postnatal mouse auditory epithelium; for these studies that take place outside of an animal's body, the animal's cochlear tissue is removed from the temporal bone and placed into a tissue culture system that follows a precise set of guidelines (e.g., the temperature of the incubator, nutrients provided to the tissue) that allows continued cellular development outside of the animal's body.

Researchers utilized each of these scientific approaches to investigate postnatal differentiation of mammalian auditory epithelium. Initial research led to the discovery that a single gene (a homolog of the *Drosophila* gene atonal) was necessary for in vivo development of hair-cells.[50] Soon after this finding, another team of researchers used an in vitro model to show that the same gene is also sufficient to develop new hair-cells.[51] The combination of these two approaches established the critical importance of this gene's expression in the developing auditory epithelium, and spurred numerous follow-up studies to determine whether the gene held the same influence in the mature mammalian cochlea.[52–58]

Through this series of additional investigations, it became increasingly apparent that the molecular and cellular environments in which this single gene is expressed can modify cell fate outcomes. Most notably, it seemed that the compelling results obtained from treatments applied during the early postnatal period start to decline in later postnatal periods,[55,56] and were not as robust in the adult organ of Corti.[53,54,57] Postnatal and adult cochlear tissues are characteristically different in their gene expression patterns; ultimately, these differences affect the ability for certain tissues to differentiate new hair-cells when given only the introduction of this particular gene. As a result, later research began to focus on which additional factors can bolster a treatment's efficacy in certain cellular environments, with specific interest in the adult mammalian auditory epithelium.

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Evaluating these early challenges in mammalian hair-cell regeneration can help us speak to the timeline of possible treatment in humans, an area that is under active investigation. With substantial achievements in the area of mammalian hair-cell regeneration, there is a clear foundation to the field that allows researchers to continue testing new approaches and refining protocols to improve treatment efficacy. Given the differences in the regenerative success observed in postnatal auditory epithelia compared with adult auditory epithelia, additional research in this field will be needed to continue informing treatment plans for human trials of hair-cell regeneration. However, lessons learned from the mammalian literature can assist with predicting the outcomes for human trials.

Perspectives on the First Human Hair-Cell Regeneration Trials

Initial outcomes will likely be blunt measures of improved hair-cell function, and refine with time and experience

Audiology patients are currently informed that their sensorineural hearing loss is permanent and that hearing aids (or cochlear implants) are their only management options. Audiologists and their patients are increasingly interested in the promise of regenerative therapies. Clinicians and researchers alike can draw on previous experience with other newly developed technologies, as well as hair-cell regeneration insights derived from animal models, to inform expectations for the trajectory of human hair-cell regenerative trials.

When considering potential outcomes for regenerative therapy for auditory hair-cells, it is critical to note that an inner hair-cell cannot properly function without the contributions of neighboring outer hair-cells and related auditory structures, including stereocilia, synapses, spiral ganglion neurons, and several other aspects of the cochlea and central auditory nervous system. Proper neural reinnervation will be needed to restore hearing function, which can occur in newly regenerated hair-cells in both avian and mammalian models.[53,54,59]

The primary outcome for initial cochlear implant trials was limited to sound awareness in the adult population. As additional electrodes and channels were added to the cochlear implant and processing strategies improved, outcomes moved beyond simple sound detection to include recognition of complex speech stimuli. Likewise, in the absence of any ability to directly image or biopsy the cochlear tissue without causing further damage, initial primary outcomes for regenerative treatments will likely be limited to increased ABR and OAE thresholds prior to improved sound detection in human trials. Future improvements to this biotechnology should then improve word recognition scores in quiet followed by improvement to speech in noise measures as the central auditory pathways reorganize in response to this renewed auditory input. As additional molecular factors inform regenerative treatment, our ability to measure the biological impact of treatment improves, and differences in underlying genetic determinants are better understood, we will advance toward applying more precise therapeutic approaches.

Etiology, Severity, and Duration of Hearing Loss are Likely to Impact Efficacy

The efficacy of regenerative therapy will be affected by the etiology of the hearing loss. Differentiating age-related hearing loss from noise-induced hearing loss is difficult with the clinical tools currently available, and many people may have a combination of age-related and noise-induced hearing loss. Age-related hearing loss is considered to be due to a change in metabolic processes of the cochlea; the aging microenvironment of the cochlea may need to be considered for its ability (or lack thereof) to support newly generated hair-cells. Furthermore, as discussed earlier, cochleae with severe noise exposure are more likely to demonstrate poor survival of supporting cells, and therefore limit regenerative potential. On the other hand, if there is a genetic etiology related to the individual's hearing loss, then it may be more efficient to use gene therapy to reintroduce that specific gene to the auditory epithelium as opposed to creating a one-size-fits-all approach to regenerating these sensory cells.

The degree of hearing loss will also likely be a significant factor in initial candidacy criteria—individuals with greater severity of hearing loss will likely be candidates prior to those with moderate hearing thresholds or better. An observation from the development of cochlear implants that may be relevant to the trajectory of regenerative trials is the difference in efficacy between patients with longstanding, untreated hearing loss and those who sought management of hearing loss through hearing aids. Cochlear implantation, as with other hearing therapies, generally yields better outcomes when the duration of auditory deprivation is minimized. Auditory deprivation leads to both peripheral[60] and central[61] neural reorganization. With the introduction of hair-cell regeneration, additional time may be needed to allow for further reorganization of these structures after restoring auditory input.

Independent of duration of hearing loss, better outcomes may also be expected from younger patient populations, as the most robust outcomes for differentiating new hair-cells in mammals are currently seen in tissues derived from younger subjects in animal studies.[55,56] Therefore, it is reasonable to hypothesize that auditory regenerative therapies will likely impart a greater effect in younger patients. The initial regeneration trials, however, will necessarily focus on the adult population where auditory outcomes are more reliably and easily measured due to their ability to understand instructions and comply with behavioral tasks. Regenerative therapies will likely extend to children only after safety and efficacy are proven in the adult patient first, as was the case for cochlear implants.[62]

Introducing potential cochlear implant candidates to regenerative therapy would result in one of three outcomes: (1) the regenerative therapy works well and audition is improved, (2) the regenerative therapy works moderately well to improve hearing but hearing aids are still necessary, or (3) regenerative therapy did not work well and the cochlear implant may still be used as an option. Ideally, regenerative therapy could replace the need for cochlear implants in a subpopulation of patients to increase frequency discrimination beyond which a cochlear implant array can

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offer; however, regenerative therapies may not be possible for all cochlear implant candidates. The mechanism by which a regenerative therapy results in increased hair-cell numbers may not be effective in all etiologies of hearing loss, and therefore may not fully replace cochlear implants. Therefore, it is likely that cochlear implants will remain a vital clinical approach due to the robust effect of applying current locally to stimulate spiral ganglion neurons.

Practical Considerations for Drug Development

Beyond determining the appropriate patient population, note that the route of administration and dosage schedule will impact the cost, tolerability, and accessibility of any potential therapeutic. There are a host of reasons why the therapy will be delivered locally to the cochlea instead of systemically via oral or intravenous administration. Importantly, the blood-labyrinthine barrier of the cochlea prevents many factors (such as medications present in the blood) from entering the cochlear endolymph. It is also possible that several therapies will need to be delivered with precise timing and doses to successfully regenerate hair-cells in the mammalian cochlea, which will necessitate more invasive, local delivery such as through the round window of the cochlea. Furthermore, systemic administration of drugs needed to stimulate hair-cell regeneration has triggered adverse events in other mammalian models.[63] Therefore, drug delivery logistics will need to be considered when designing the therapeutic drug as well as the clinical trial itself.

Regenerative Therapy Is Promising, but Years of Research Will Be Required before Becoming a Routine Therapeutic Option

The efficacy of regenerative therapies is likely to improve with additional refinement and experience after it is initially introduced to the adult human inner ear. Although years of additional research will be required before regenerative therapies can be introduced as a routine option for clinical care, the field as a whole shows great promise as we work to increase audibility for those who struggle with the limitations of currently available technologies. As audibility increases with the success of hair-cell regeneration trials, devices such as hearing aids or noise cancellation technologies may also assist those patients who demonstrate incomplete hair-cell regeneration.

Audiologists will remain a critical part of tracking the trajectory of patients who undergo regenerative therapies. While objective measures such as ABRs and OAEs will be a standard approach to capture improvements in function after delivering the therapy, these measures may not capture the full experience from an individual. Particularly during early phases of regenerative trials, an audiologist will be needed to help translate the patient's report of change in audition and sound quality to the medical team who is administering the regenerative therapy. Audiologists and otologists will need to work as a team to ensure optimal short-term and long-term outcomes for patients who choose regenerative therapies for hearing loss.

Conflict of Interest
None declared.

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References

1 Eggermont JJ. Hearing Loss: Causes, Prevention, and Treatment. London, UK: Academic Press 2017
2 Musiek FE, Baran JA. The Auditory System: Anatomy, Physiology and Clinical Correlates. Boston, MA, USA: Allyn Bacon 2007
3 Wu P-Z, Wen W-P, O'Malley JT, Liberman MC. Assessing fractional hair-cell survival in archival human temporal bones. Laryngoscope 2020;130(02):487–495
4 Kochkin S. MarkeTrak VII. Hear J 2005;58(09):30. https://journals.lww.com/thehearingjournal/Fulltext/2005/09000/MarkeTrak_VII_Customer_satisfaction_with_hearing.8.aspx
5 Keithley EM. Pathology and mechanisms of cochlear aging. J Neurosci Res 2020;98(09):1674–1684
6 Brignull HR, Raible DW, Stone JS. Feathers and fins: non-mammalian models for hair-cell regeneration. Brain Res 2009;1277:12–23
7 Dooling RJ, Ryals BM, Manabe K. Recovery of hearing and vocal behavior after hair-cell regeneration. Proc Natl Acad Sci U S A 1997;94(25):14206–14210
8 Ryals BM, Dent ML, Dooling RJ. Return of function after hair-cell regeneration. Hear Res 2013;297:113–120
9 Tucci DL, Rubel EW. Physiologic status of regenerated hair-cells in the avian inner ear following aminoglycoside ototoxicity. Otolaryngol Head Neck Surg 1990;103(03):443–450
10 Woolley SM, Wissman AM, Rubel EW. Hair cell regeneration and recovery of auditory thresholds following aminoglycoside ototoxicity in Bengalese finches. Hear Res 2001;153(1-2):181–195
11 Holt JR. Vertebrate Hair-Cells. Springer Handbook of Auditory Research Vol. 27New York: Springer; 2006
12 Ashmore J, Avan P, Brownell WE, et al. The remarkable cochlear amplifier. Hear Res 2010;266(1-2):1–17
13 Brownell WE. Outer hair-cell motility and cochlear frequency selectivity. Auditory Frequency Selectivity 1986. Boston, MA: Springer
14 Dallos P, Wu X, Cheatham MA, et al. Prestin-based outer hair-cell motility is necessary for mammalian cochlear amplification. Neuron 2008;58(03):333–339
15 Russell IJ, Sellick PM. Intracellular studies of hair-cells in the mammalian cochlea. J Physiol 1978;284(01):261–290
16 Cheng AG, Cunningham LL, Rubel EW. Mechanisms of hair-cell death and protection. Curr Opin Otolaryngol Head Neck Surg 2005;13(06):343–348
17 Moore BCJ. Dead regions in the cochlea: diagnosis, perceptual consequences, and implications for the fitting of hearing AIDS. Trends Amplif 2001;5(01):1–34
18 Bhatt KA, Liberman MC, Nadol JB Jr. Morphometric analysis of age-related changes in the human basilar membrane. Ann Otol Rhinol Laryngol 2001;110(12):1147–1153
19 Nadol JB Jr, Ketten DR, Burgess BJ. Otopathology in a case of multichannel cochlear implantation. Laryngoscope 1994;104(3 Pt 1):299–303

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20 Pickles JO, Comis SD, Osborne MP. The morphology of stereocilia and their cross-links in relation to noise damage in the guinea pig. In: Basic and Applied Aspects of Noise-Induced Hearing Loss 1986:31–41. Boston, MA: Springer

21 Tsuprun V, Schachern PA, Cureoglu S, Paparella M. Structure of the stereocilia side links and morphology of auditory hair bundle in relation to noise exposure in the chinchilla. J Neurocytol 2003;32 (09):1117–1128

22 Cotanche DA, Dopyera CE. Hair cell and supporting cell response to acoustic trauma in the chick cochlea. Hear Res 1990;46(1-2):29–40

23 Cotanche DA, Lee KH, Stone JS, Picard DA. Hair cell regeneration in the bird cochlea following noise damage or ototoxic drug damage. Anat Embryol (Berl) 1994;189(01):1–18

24 Girod DA, Tucci DL, Rubel EW. Anatomical correlates of functional recovery in the avian inner ear following aminoglycoside ototoxicity. Laryngoscope 1991;101(11):1139–1149

25 Richardson GP, Russell IJ. Cochlear cultures as a model system for studying aminoglycoside induced ototoxicity. Hear Res 1991;53 (02):293–311

26 Lippe W, Rubel EW. Development of the place principle: tonotopic organization. Science 1983;219(4584):514–516

27 Rebillard G, Ryals BM, Rubel EW. Relationship between hair-cell loss on the chick basilar papilla and threshold shift after acoustic overstimulation. Hear Res 1982;8(01):77–81

28 Rubel EW, Ryals BM. Development of the place principle: acoustic trauma. Science 1983;219(4584):512–514

29 Rubel EW, Lippe WR, Ryals BM. Development of the place principle. Ann Otol Rhinol Laryngol 1984;93(6 Pt 1):609–615

30 Ryals BM, Rubel EW. Patterns of hair-cell loss in chick basilar papilla after intense auditory stimulation. Frequency organization. Acta Otolaryngol 1982;93(1-6):205–210

31 LeMasurier M, Gillespie PG. Hair-cell mechanotransduction and cochlear amplification. Neuron 2005;48(03):403–415

32 Beurg M, Tan X, Fettiplace R. A prestin motor in chicken auditory hair-cells: active force generation in a nonmammalian species. Neuron 2013;79(01):69–81

33 Hudspeth AJ, Choe Y, Mehta AD, Martin P. Putting ion channels to work: mechanoelectrical transduction, adaptation, and amplification by hair-cells. Proc Natl Acad Sci U S A 2000;97(22):11765–11772

34 Ryals BM, Rubel EW. Ontogenetic changes in the position of hair-cell loss after acoustic overstimulation in avian basilar papilla. Hear Res 1985;19(02):135–142

35 Cotanche DA. Regeneration of hair-cell stereociliary bundles in the chick cochlea following severe acoustic trauma. Hear Res 1987;30(2-3):181–195

36 Cruz RM, Lambert PR, Rubel EW. Light microscopic evidence of hair-cell regeneration after gentamicin toxicity in chick cochlea. Arch Otolaryngol Head Neck Surg 1987;113(10):1058–1062

37 Corwin JT, Cotanche DA. Regeneration of sensory hair-cells after acoustic trauma. Science 1988;240(4860):1772–1774

38 Ryals BM, Rubel EW. Hair cell regeneration after acoustic trauma in adult Coturnix quail. Science 1988;240(4860):1774–1776

39 Manley GA, Schulze M, Oeckinghaus H. Otoacoustic emissions in a song bird. Hear Res 1987;26(03):257–266

40 Froymovich O, Rebala V, Salvi RJ, Rassael H. Long-term effect of acoustic trauma on distortion product otoacoustic emissions in chickens. J Acoust Soc Am 1995;97(5 Pt 1):3021–3029

41 Adler HJ, Kenealy JFX, Dedio RM, Saunders JC. Threshold shift, hair-cell loss, and hair bundle stiffness following exposure to 120 and 125 dB pure tones in the neonatal chick. Acta Otolaryngol 1992;112(03):444–454

42 Umemoto M, Sakagami M, Ashida K, Fukazawa K, Matsunaga T, Senda T. Regeneration of adult budgerigar's hair-cells following acoustic trauma. ORL J Otorhinolaryngol Relat Spec 1993;55(06):325–331

43 Woolley SM, Rubel EW. Vocal memory and learning in adult Bengalese finches with regenerated hair-cells. J Neurosci 2002; 22(17):7774–7787

44 Marean GC, Burt JM, Beecher MD, Rubel EW. Hair cell regeneration in the European starling (*Sturnus vulgaris*): recovery of pure-tone detection thresholds. Hear Res 1993;71(1-2):125–136

45 Dooling RJ, Ryals BM, Dent ML, Reid TL. Perception of complex sounds in budgerigars (*Melopsittacus undulatus*) with temporary hearing loss. J Acoust Soc Am 2006;119(04):2524–2532

46 Marean GC, Burt JM, Beecher MD, Rubel EW. Auditory perception following hair-cell regeneration in European starling (*Sturnus vulgaris*): frequency and temporal resolution. J Acoust Soc Am 1998;103(06):3567–3580

47 Cafaro J, Lee GS, Stone JS. Atoh1 expression defines activated progenitors and differentiating hair-cells during avian hair-cell regeneration. Dev Dyn 2007;236(01):156–170

48 Duncan LJ, Mangiardi DA, Matsui JI, Anderson JK, McLaughlin-Williamson K, Cotanche DA. Differential expression of unconventional myosins in apoptotic and regenerating chick hair-cells confirms two regeneration mechanisms. J Comp Neurol 2006; 499(05):691–701

49 Roberson DW, Alosi JA, Cotanche DA. Direct transdifferentiation gives rise to the earliest new hair-cells in regenerating avian auditory epithelium. J Neurosci Res 2004;78(04):461–471

50 Bermingham NA, Hassan BA, Price SD, et al. Math1: an essential gene for the generation of inner ear hair-cells. Science 1999;284 (5421):1837–1841

51 Zheng JL, Gao WQ. Overexpression of Math1 induces robust production of extra hair-cells in postnatal rat inner ears. Nat Neurosci 2000;3(06):580–586

52 Atkinson PJ, Wise AK, Flynn BO, Nayagam BA, Richardson RT. Hair cell regeneration after ATOH1 gene therapy in the cochlea of profoundly deaf adult guinea pigs. PLoS One 2014;9(07):e102077

53 Kawamoto K, Yagi M, Stöver T, Kanzaki S, Raphael Y. Hearing and hair-cells are protected by adenoviral gene therapy with TGF-beta1 and GDNF. Mol Ther 2003;7(04):484–492

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

55 Kelly MC, Chang Q, Pan A, Lin X, Chen P. Atoh1 directs the formation of sensory mosaics and induces cell proliferation in the postnatal mammalian cochlea in vivo. J Neurosci 2012;32(19):6699–6710

56 Liu Z, Dearman JA, Cox BC, et al. Age-dependent in vivo conversion of mouse cochlear pillar and Deiters' cells to immature hair-cells by Atoh1 ectopic expression. J Neurosci 2012;32(19):6600–6610

57 Shou J, Zheng JL, Gao WQ. Robust generation of new hair-cells in the mature mammalian inner ear by adenoviral expression of Hath1. Mol Cell Neurosci 2003;23(02):169–179

58 Staecker H, Schlecker C, Kraft S, Praetorius M, Hsu C, Brough DE. Optimizing atoh1-induced vestibular hair-cell regeneration. Laryngoscope 2014;124(Suppl 5):S1–S12

59 Ryals BM, Westbrook EW. TEM analysis of neural terminals on autoradiographically identified regenerated hair-cells. Hear Res 1994;72(1-2):81–88

60 Liberman MC, Kiang NY. Acoustic trauma in cats. Cochlear pathology and auditory-nerve activity. Acta Otolaryngol Suppl 1978;358:1–63

61 Cardon G, Campbell J, Sharma A. Plasticity in the developing auditory cortex: evidence from children with sensorineural hearing loss and auditory neuropathy spectrum disorder. J Am Acad Audiol 2012;23(06):396–411, quiz 495

62 Waltzman SB, Shapiro WH. Cochlear implants in children. Trends Amplif 1999;4(04):143–162

63 Mizutari K, Fujioka M, Hosoya M, et al. Notch inhibition induces cochlear hair-cell regeneration and recovery of hearing after acoustic trauma. Neuron 2013;77(01):58–69

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