ABSTRACT: In more than 80% of cases of hearing loss, the cause is directly or indirectly related to the degeneration and death of sensory hair cells and their associated spiral ganglion neurons (M. C. Holley, 2002; J. F. Willot, 1991). Although certain animal species have the ability to regenerate lost hair cells, humans do not. If regeneration of these hair cells or the generation of new hair cells became possible, then many individuals would be given the potential to restore hearing. Several laboratories around the world are currently attempting to understand the molecular factors and mechanisms necessary for cochlear hair cell function, death, and (re)generation in the animal and human species. Three leading approaches—gene therapy, cell transplant, and drug delivery—are making rapid advances in generating new or restored hair cells in the human cochlea. This article includes a description of cochlear hair cell functioning, the history of hair cell regeneration efforts, and a comparison of approaches to cochlear hair cell regeneration.

KEY WORDS: cochlear hair cells, drug delivery, gene therapy, hair cell regeneration, sensorineural hearing loss, stem cells

A Review of Past and Present Hair Cell Regeneration Techniques

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Hair cells are the sensory receptors that are situated on the fibrous basilar membrane in the organ of Corti (Martin & Clark, 2003; Newby & Popelka, 1992; Sataloff, 1966; Willot, 1991). There are two types of sensory hair cells: inner and outer hair cells. The inner hair cells are arranged in a single row in the organ of Corti; the outer hair cells are arranged in three parallel rows (Martin & Clark, 2003; Newby & Popelka, 1992; Sataloff, 1966). The inner hair cells are round and squat; the outer hair cells are longer and slimmer. These two types of hair cells have very different roles in the hearing process. Inner hair cells are the major site of the transduction of mechanical energy to neural electrical signals. Outer hair cells, however, appear to also impact the sensitivity of the cochlea.

Loss of the inner hair cells in a region of the cochlea would be expected to eliminate the region’s participation in hearing, while loss of the outer hair cells would have less devastating effects on cochlear input to the brain, but might alter properties of the cochlea in ways that would significantly elevate thresholds. (Willot, 1991, p. 20)

The inner hair cells contain some 3,000 to 3,500 ciliated cells, and the outer cells contain from 9,000 to 12,000 ciliated cells. The inner and outer hair cells run in parallel rows. They are connected to some 24,000 transverse nerve fibers leading to the central core of the cochlea, the modiolus (Newby & Popelka, 1992; Sataloff, 1966). These nerve fibers form the cochlear branch of cranial nerve VIII. The cochlear branch joins the vestibular branch and forms cranial nerve VIII, the auditory or vestibulocochlear nerve (Martin & Clark, 2003; Newby & Popelka, 1992; Sataloff, 1966). This nerve proceeds to the nuclei in the brainstem and transmits its electrical signals to the brain. Overall, cochlear hair cells primarily function to convert auditory stimuli into electrical signals, and the vestibular hair cells primarily function to help detect changes in body and head positions so that information can be sent and processed in the brain.

Each human is born with approximately 3,000 to 3,500 inner hair cells and 9,000 to 12,000 outer hair cells (Newby & Popelka, 1992; Northern & Downs, 2002). No new hair cells are able to develop after birth, and damaged cells are unable to repair themselves (Martin & Clark, 2003; Northern & Downs, 2002). However, sensory systems
do exhibit some neural plasticity, which is the capacity to functionally rewire neural connection patterns (National Organization for Hearing Research Foundation, 2002). These systems are not fully mature at birth, and some considerable modification can take place postnatally. However, neural plasticity begins to diminish as one ages (Martin & Clark, 2003; Newby & Popelka, 1992; Willot, 1991). In addition, there are several other etiologies for hair cell damage or loss, which result in sensorineural hearing loss.

Etiologies of Hair Cell Damage

Cochlear damage and disease lead to the most common type of hearing disorder, called sensorineural hearing loss. Sensory implies damage to the cochlea, or more typically, damage or death of the hair cells. Neural implies damage to the auditory nerve or nerve fibers in or beyond the region of the cochlea. In general, the most common cause of hearing loss involves damage or death of hair cells in the cochlea’s organ of Corti (Martin & Clark, 2003; Newby & Popelka, 1992; Sataloff, 1966; Willot, 1991).

Mechanisms of Hair Cell Death

There are two general ways that hair cells die in the body: necrosis and apoptosis. “Necrosis cell death is a passive process characterized by rupture of cell membrane and spilling of the cell’s cytoplasmic contents into the surrounding tissue” (Ryals & Cunningham, 2003, p. 104). This death is not a natural process of the body, but rather is induced by trauma or disease. Necrotic death occurs less frequently than apoptotic cell death. Apoptosis occurs because each cell has a built-in program of cellular death (Raphael, 2002; Ryals & Cunningham, 2003). It is a natural progression of regulated and organized development, similar to the mitotic cell birth process, in order to maintain proper cell performance in the body. Overall, apoptosis functions to eliminate damaged or unnecessary cells without changing the surrounding healthy tissue. Research has shown that cells undergo apoptosis as a result of presbycusis and after noise-induced hair cell loss (Raphael, 2002; Ryals & Cunningham, 2003).

In mammals, loss of cochlear hair cells leads to permanent hearing impairments and/or balance disorders. However, within the past 40 years, researchers have gained considerable hope that someday, damaged or dead hair cells can be regenerated or new hair cells can be generated. Thus, individuals with sensory hearing loss may have the potential to regain normal hearing sensitivity.


The interest in hair cell regeneration began in the 1970s when researchers discovered that fish, amphibians, and other cold-blooded animals continuously grow new hair cells throughout their lives (Corwin & Oberholtzer, 1997). All of these species have highly evolved auditory centers with many characteristics that are similar to the mammalian ear. Thus, these species are important models to help researchers understand the structure and function of the mammalian auditory system (Corwin & Oberholtzer, 1997).

Specifically, researchers can study the degeneration of hair cells, spontaneous generation of new hair cells, or regeneration of existing hair cells in these species to gain an understanding of the molecular factors and mechanisms involved in these processes (Raphael, 2002; Ryals & Cunningham, 2003). With that knowledge, attempts can be made to mimic these factors and processes in the organ of Corti of mammals in hopes of regenerating old or generating new hair cells.

Fish

Fish use two systems, the inner ear and the lateral line, to detect sounds and vibrations, respectively, which allow them to experience their aquatic environment (Lowenstein, 1957; Schellart & Wubbels, 1998; Walshe, Walsh, & McConn, 2003). Because the bodies of fish are similar to the density of seawater, sound waves cause the entire fish to move with the water and the sound passes through their bodies (Lowenstein, 1957; Schellart & Wubbels, 1998; Walshe et al., 2003). The otoliths, the bones of the inner ear of the fish, are composed of calcium carbonate and differ in dimension and shape among the various species of fish (Northern & Downs, 2002). Regardless of the species, the otoliths are much denser and thus move slower than the rest of the fish (Lowenstein, 1957; Schellart & Wubbels, 1998; Walshe et al., 2003). The difference between the motion of the otoliths and the motion of the fish stimulates the cilia on the hair cells, which is interpreted as sound (Lowenstein, 1957; Schellart & Wubbels, 1998; Walshe et al., 2003).

The lateral line system extends along the side of the fish’s body and onto the head, which permits the fish to detect acoustic signals over a distance of one to two body lengths and at low frequencies (Lowenstein, 1957; Schellart & Wubbels, 1998). Neuromasts, the mechanoreceptive organs of the lateral line system, are composed of bundles of sensory and supporting cells. Projecting from these cells are hair cells that are enclosed in a gelatinous cover, which are continuously firing neural signals (Northern & Downs, 2002). The pressure of the water in the fish’s environment stimulates the neuromasts to move, which causes the hair cells to bend. Depending on the direction of the hair cells, the nerve impulses get either enhanced or depressed. Fish use this lateral line system to detect the relative motion between themselves and the surrounding water in order to identify their orientation in the water and to avoid collisions and predation (Lowenstein, 1957; Schellart & Wubbels, 1998; Walshe et al., 2003).

In an often cited study, Walshe et al. (2003) focused on the fully blind cavefish, which relies solely on its neuromasts to compensate for total blindness in an environment where no light exists. The lateral line sensory systems allow the cavefish to detect objects and vibrations (Walshe et al., 2003). The neuromasts in these fish are known to be able to regenerate hair cells that have been lost (Lowenstein, 1957;
The middle ear chamber is located beyond the TM; it carries sound waves toward the tympanic membrane (TM) via the outer ear via the acoustic meatus, which is a tube that reflects sound and acts as a sound funnel. The sound enters feathers that, like the human pinna, protect the opening. The bird lacks a pinna and is hidden by specialized auricle of hearing and balance. Like human ears, the bird ear is involved in generating these additional hair cells, researchers have gained much knowledge about how to potentially replicate this process in the human ear.

**Amphibians**

Regeneration of hair cells in the inner ear is also observed in high-order amphibrans (Walshe et al., 2003; Wever, 1985). Like the cavefish, amphibians possess the lateral line system and can also regenerate sensory hair cells (Wever, 1985). Bullfrogs that have been treated with an aminoglycoside, a certain type of antibiotic that destroys hair cells, regenerates lost sensory cells in their vestibular systems. Interestingly enough, the regenerated hair cells developed an active afferent nerve supply, which supports the notion that their sensory organ had recovered its function (Walshe et al., 2003).

Hair cells in both the inner ear and the lateral line in fish and amphibians are continuously produced from supporting cells undergoing mitosis (Walshe et al., 2003). These findings indicate that fish and amphibians have a distinct advantage over mammals due to their ability to self-repair damaged or dead hair cells.

**Sharks**

Whereas fish and amphibrans produce sensory cells throughout life to self-repair damaged cells, sharks possess a special characteristic in that they have hundreds of thousands of hair cells added after embryonic development (Reef Quest Centre for Shark Research, 2003; Walshe et al., 2003). In fact, “the inner ear of sharks, which were once considered primitive, actually have far more sensory cells than the human inner ear, because they are able to add new hearing and balance cells throughout life” (Corwin & Oberholtzer, 1997, p. 951). A juvenile shark has approximately 20,000 hair cells, and an adult eventually generates 240,000 hair cells (Corwin, 2003). This hair cell increase allows improved hearing sensitivity as the shark matures (Reef Quest Centre for Shark Research, 2003; Walshe et al., 2003). By assessing the genes and mechanisms involved in generating these additional hair cells, researchers have gained much knowledge about how to potentially mimic this process in the human ear.

**Birds**

The avian (bird) ear, similar to the human ear, is an organ of hearing and balance. Like human ears, the bird ear is made up of three parts: the outer, middle, and inner chamber (Earth-Life Web, 2005; Pesek, 2003). The outer ear lacks a pinna and is hidden by special auricle feathers that, like the human pinna, protect the opening, reflect sound, and act as a sound funnel. The sound enters the outer ear via the acoustic meatus, which is a tube that carries sound waves toward the tympanic membrane (TM). The middle ear chamber is located beyond the TM; is air filled; and contains muscles, ligaments, and a cochlear (round) window. Also, avian ears have one ossicular bone known as the columella. The columella functions to pick up sound vibrations from the TM and transmit them to the oval window in the inner ear. The inner ear is bathed in fluid and consists of a complex structure known as the membranous labyrinth. It serves as the sensory portion of the inner ear and includes the vestibular labyrinth and the cochlear labyrinth (Earth-Life Web, 2005; Pesek, 2003).

Hearing in birds is primarily dependent on the organ of Corti, which is located in the cochlear labyrinth. This organ contains sensitive hair cells that respond to vibrations from the columella that is connected to the oval window. These hair cells then transmit the impulses to the brain, where the sound can be interpreted. Overall, there is a strong similarity between the human and avian ear anatomy and physiology; therefore, understanding and using avian models are thought to be important to hair cell regeneration efforts in humans.

Regeneration of hair cells in the avian species was first reported in 1987 (Corwin & Cotanche, 1988), when researchers noticed immature-looking hair cells after noise-induced hair cell loss (Stone & Rubel, 2000; Walshe et al., 2003). Researchers then destroyed the hair cells of birds using aminoglycoside-induced treatments. Subsequently, these hair cells regenerated and became functional again (Corwin & Cotanche, 1988; Society for Neuroscience, 1994; Stone & Rubel, 2000; Walshe et al., 2003). These hair cell regeneration discoveries in warmblooded species following exposure to ototoxic drugs or intense acoustic stimulation have changed the view on how hearing and balance may someday be treated. Additionally, this knowledge stimulated a new wave of research, which had and still has three primary goals: first, a greater understanding of hair cell regeneration in the inner ear of birds and other nonmammalian vertebrates; second, awareness of the functional capabilities of the inner ear and neural pathways following regeneration; and last, the ability to stimulate replacement of lost or injured cells in the inner ear of mammals (Bermingham-McDonogh & Rubel, 2003).

The avian model became and is still one of the most popular models of study for hair cell regeneration. Research on various avian species has shown that structural hair cell recovery is faster than functional hair cell recovery (Walshe et al., 2003). Additional studies on avian species have uncovered that certain growth factors, receptors, and proteins, in addition to retinoic acid, all have critical roles in the initial growth and the regeneration processes of their sensory cells (Oesterle, Tsue, & Rubel, 1997; Stone & Rubel, 2000; Witte, Montcouquipol, & Corwin, 2001). Many molecular markers that stimulate and inhibit regeneration have been uncovered. Finally, avian research has prompted researchers to identify the specific differences between human hair cells and bird hair cells, despite all the commonalities (Bermingham-McDonogh & Rubel, 2003; Stone & Rubel, 2000; Walshe et al., 2003). This knowledge is critical because it is the differences between the two species that limits the ability to trigger new hair cell growth in humans.
Mammals

Researchers have studied the auditory processes of a broad range of mammals ranging from mice to whales. Mammals do not have spontaneous regeneration of hair cells. Although there are slight differences between the ears of different mammal species, the process or mechanism at work is similar. By analyzing mammalian hair cell anatomy and physiology, hair cell growth and function, and hair cell death, researchers hope to determine the proteins, genes, receptors, chemicals, and mechanisms needed for such processes (Corwin & Oberholtzer, 1997; Walshe et al., 2003). That information can then be used to identify what results when certain genes, proteins, or chemicals are added, inhibited, or substituted (Corwin & Oberholtzer, 1997; Walshe et al., 2003). Mammalian findings can then be compared to species that can regenerate their hair cells, such as birds, fish, and sharks.

A few studies using mammalian models have helped to uncover some of the important factors that determine cell differentiation, proliferation, maintenance, and fate (Ryals & Cunningham, 2003). Forge, Li, Corwin, and Nevill (1993) indicated that mammals have the capacity for hair cell regeneration. After using scanning electron micrographs to examine the utricles of the inner ears of guinea pigs that had been subjected to ototoxic drug treatments, Forge found that hair cells reappeared 4 weeks after completion of the ototoxic application (Forge et al., 1993). Similar growth was observed in the cristae and saccules of the guinea pigs, which suggested that these phenomena occur throughout the mammalian vestibular system (Forge et al., 1993).

In addition, retonic acid has been found to stimulate the regeneration of hair cells on the cochleas of neonatal rats following ototoxic poisoning (Chardin et al., 1995). Retonic acid is a vitamin A derivative that is critical to differentiation and maturity (Ryals & Cunningham, 2003). The growth factors and receptors from seven different protein families in the cochlear tissues of the juvenile rat were examined and showed that a variety of known growth factors play significant roles in the development, maintenance, and repair of the inner ear (Malgrange et al., 1997).

Other investigations have shown that mice that are deficient in the gene Math1 do not develop fully differentiated hair cells in the cochlea (Bermingham et al., 1999). In addition, studies on chicks have revealed that the genes Notch1 and Delta1 also mediate cell specification in several tissues (Ryals & Cunningham, 2003). These findings reinforce the possibility that human hair cells can be coaxed to regenerate using growth factors or other molecules. Overall, these past mammalian studies have paved the way for current regeneration approaches that are being attempted on similar laboratory animals.

**RECENT APPROACHES IN HAIR CELL GENERATION: 1999–2005**

**Gene Therapy**

*Characteristics.* “Gene therapy is defined as the introduction of genetic materials into cells to bring about a therapeutic effect” (Martin & Drew, 1999, p. 1). The use of gene therapy as a potential treatment for disease has been experimented with since the 1970s (Valere, 1999). This therapeutic approach attempts to correct the damage or disease at the specific source (or cause) of the dysfunction of a gene or protein, with little or no side effects (Drew & Martin, 1999). However, before such therapy can become a clinical reality, many obstacles must be overcome, newer technology needs to be developed, and proper vector systems must be identified (Drew & Martin, 1999).

“The ultimate goal is long-term, sufficiently regulated expression of the transferred gene, achieved by a single, lifetime treatment involving a simple noninvasive, safe, and efficient gene delivery which can be incorporated in clinical practice” (Murphy, 1999, p. 22). The first important step in gene therapy is to design a safe and effective delivery vector that can enter target cells and release appropriate levels of gene expression over a period of time (Holley, 2002). Viruses have the potential of meeting all of these criteria, although no one viral system currently meets all of them (Murphy, 1999). The viruses that are currently being highly researched and used as gene therapy vehicles are retroviruses (RVs), adenoviruses (AdVs), herpes viruses (HV), and adeno-associated viruses (AAVs) (Murphy, 1999; Panno, 2005).

First and foremost, the major problem with gene therapy lies with selection of the viral vectors (Murphy, 1999). Viral vectors are problematic because viruses have the ability to replicate their own viral genome (Panno, 2005). The immune system currently poses another obstacle to the efficacy of most recombinant viral vectors because its primary function is to eliminate viral infections and foreign bodies in the human system (Holley, 2002; Murphy, 1999). However, techniques are being developed to reduce, block, or overcome these challenges (Murphy, 1999).

Researchers are also attempting to use nonviral vectors. However, these do not currently seem promising as a choice of delivery system because they are less effective and less specific than viral delivery systems. Examples of nonviral vectors include plasmids, liposomes, synthetic polymer, electroporation, and biliotics (Holley, 2002; Miller, 1999). The process of developing nonviral delivery systems is still in infancy, but someday the use of nonviral delivery systems may surpass or rival the use of viral delivery systems in the gene therapy approach (Miller, 1999).

Targeting a specific site within the body presents another challenge to the gene therapy approach (Martin, 1999). Targeting vectors can be achieved in a multitude of ways that use differing levels of sophistication. Targeting can occur by simply applying the vector directly to the required site of action or by using genetic engineering to direct the vector components to recognize and bind to specific proteins or receptor sites in the target cells. However, no clear targeting strategy has been shown to be most effective for all purposes. Rather, targeting appears to be tailored to the specific target region or disease. With intensive research being conducted on viral life cycles, protein engineering, and molecular pathways, there will soon be a clearer model for an ultimate gene delivery system (Martin, 1999).
**Gene therapy and the inner ear.** Interest in using gene therapy for regenerating sensory cells began in 1995 (Drew & Martin, 1999). The inner ear is an attractive target for gene therapy for three main reasons. First, the ear has a small and localized population of cells in comparison with other regions of the body. Second, the inner ear is fairly isolated from other cells due to the presence of the otic capsule, which seals off the inner ear space. Finally, the presence of perilymphatic and endolymphatic fluid-filled spaces allows the diffusion of vectors into areas other than the direct site of inoculation (Ishimoto, Kowamoto, Kanzaki, & Raphael, 2002).

“Cells within the ear can be modified to secrete therapeutic gene products, such as growth factor, and they can be re-programmed to develop different functional properties or to correct an inherent genetic defect” (Holley, 2002, p. 163). Gene insertion involves the introduction of new DNA into the system either by direct injection of DNA or through viral vectors (Forge et al., 1993). Currently, the most specific and efficient vehicle for delivery to the inner ear is the adenoviral vector (Hackett & Crystal, 2000; Holley, 2002).

In recent years, a number of studies on the application of gene therapy to the auditory system have been conducted. In 1996, Raphael, Frisancho, and Roesessler demonstrated that once inserted into the cochlea, adenoviral vectors did not cause any major morphological signs of pathology or toxicity. Lalwani and colleagues (Lalwani, Walsh, Carvalho, Muzyczka, & Mhatre, 1998; Lalwani, Walsh, Reilly, Muzyczka, & Mhatre, 1996) found that the AAV transfer gene could persist in the cochlea and in the vestibular neuroepithelia of guinea pigs for up to 6 months. They suggested that this gene might be useful for clinical treatment. Another critical finding occurred in 1999, when Bermingham and colleagues uncovered that the Math1 gene was essential for genesis of hair cells in the inner ear (Bermingham et al., 1999).

The first major research breakthrough in inner ear gene therapy occurred in 2000. Researchers at Genentech in South San Francisco reported growing new hair cells in inner ear tissue taken from newborn rats using the Math1 gene to promote sensory cell growth (Seppa, 2000; Travis, 2003; Zheng & Gao, 2000). In the developing human fetus, the protein encoded by Math1 induces up to 16,000 cells in the cochlea (Seppa, 2000). However, once this population of cells appeared, Math1 seemed to shut off (Seppa, 2000). Using this knowledge, researchers exposed cells from a rat cochlea to a DNA plasmid containing the Math1 gene and jotted the recipient cell with an electric shock to facilitate entry of the plasmid. To track the plasmid, a green fluorescent protein was encoded in the structure of the newly inserted gene. Within 6 to 12 days, the plasmids induced the cells of the cochleas of the newborn rats to produce the Math1 protein. The addition of the Math1 protein resulted in the regeneration of hundreds of hair cells (Seppa, 2000; Zheng & Gao, 2000).

Raphael and Kawamoto were among the first to induce the production of thousands of new hair cells in the mammalian cochlea by inserting the gene Atoh1, also known as Math1, via an adenoviral vector, a safe cell-type specific carrier used to insert genetic material, into the supporting cells of live guinea pigs (Izumikawa et al., 2005). These new hair cells exhibited the characteristic morphology of a hair cell, were ectopically positioned, and appeared to attract auditory nerve fibers (Kawamoto, Ischimoto, Minoda, Brough, & Raphael, 2003). Thus, the new hairs appeared functional. However, in this experiment, the animals were not deaf. Thus, in 2004–2005, Raphael and Kawamoto repeated their experiment after first destroying the hair cells in both ears of the 10 guinea pig subjects. Three days later, the hair cells were confirmed to be dead. On the fourth day, gene therapy was used to insert the Atoh1 gene via an adenoviral vector into the left cochlea of each guinea pig. After approximately 2 months, the appearance of new hair cells in the left ear was noted; the right (untreated control) ear had no new growth. Auditory brainstem response testing showed that the auditory nerve was functional. Therefore, this experiment showed that the insertion of a gene successfully regrew functional hair cells in a guinea pig (Izumikawa et al., 2005).

Gene therapy is thought to have a promising future. However, there are still numerous technological and scientific limitations as well as ethical and social concerns that must be addressed before clinical application in humans can occur. One such problem is the short-lived nature of gene therapy. “Sustaining any long-term benefits from DNA integrated into the body is difficult because of the rapid dividing nature of many cells” (Vargo, 2004, p. 2). Therefore, numerous rounds of gene therapy would be needed to produce significant changes in a human cochlea. Also, immune response may create problems because the body recognizes the inserted genes as foreign substances (Holley, 2002; Murphy, 1999). Viral vectors may also generate immune and inflammatory responses, resulting in difficulties in gene control and in targeting specific tissues, and the viruses may retain, acquire, or recover pathogenicity (the ability to cause disease) (Vargo, 2004). In addition, there are often multiple genes that are responsible for a particular disease or disorder; therefore, inserting a single transgene may not work (Nossal & Coppel, 1989; Panno, 2005). Overall, gene therapy in the inner ear region is relatively less controversial than germ-line gene therapy because gene therapy only involves somatic cells and not the gametes or pre-embryos that are used in germ-line gene therapy (Sade & Khushf, 1998). All of these factors further limit the future outlook for gene therapy as a regeneration approach.

**Cell Transplantation**

**Characteristics.** Cell transplant is another potential approach for the treatment of cell or tissue damage, death, or disorder. This approach gained much interest when Thomson first isolated a human embryonic stem cell (hESC) (Thomson et al., 1998). There are different types of stem cells, but all types share two basic characteristics. First, all stem cells can continue to grow and proliferate for long periods of time, thus constantly maintaining a pool of cells available for use (Commission on Life Sciences, 2002; Condie, 2002; Mackay-Sim, 2004; Prentice, 2003). Second, given the correct signals, such as growth hormone,
steroid hormone, or insulin, stem cells can differentiate into any type of cell in the body (Commission on Life Sciences, 2002; Malgrange et al., 1997; Prentice, 2003).

The stem cell goes through multiple stages in development. First, the cell is immature and unspecialized. Then, the cell forms into a precursor for a specific cell type and becomes a progenitor cell. Last, the cell becomes a differentiated cell when it develops into a fully specialized cell that carries a specific function in the body. Stem cells also differ in their potencies or their flexibility in their possibilities to differentiate. A unipotent cell can only differentiate into one cell type, whereas a multipotent cell can differentiate into several different cell and tissue types. Also, a pluripotent stem cell can develop into almost any cell type in the adult body. Totipotent cells are similar to pluripotent cells, but can also form embryonic cells and tissues (Prentice, 2003).

Embryonic stem cells. Although the human body is made up of different types of cells, all of these different types initially arise from a single cell, the fertilized egg. These cells are called ESCs and were first isolated from mouse embryos by two independent groups in 1981 (Evans & Kaufman, 1981; Martin, 1981) and from human embryos in 1998 (Evans & Kaufman, 1981; Martin, 1981; Thomson et al., 1998). These ESCs are those that continually self-renew until signaled to become a specialized cell in the body (Commission on Life Sciences, 2002; Prentice, 2003). They must be halted in their early development when the cells have divided to form approximately 1,000 cells in order to prevent against creating an individual (Pecorino, 2001).

These cells are advantageous because of their high potency and their potential to become any cell in the body (Commission on Life Sciences, 2002; Condiac, 2002; Mackay-Sim, 2004; Pecorino, 2001). Also, they are immortal because one cell line can potentially supply endless amounts of cells with carefully defined characteristics (Commission on Life Sciences, 2002; Condiac, 2002; Mackay-Sim, 2004; Pecorino, 2001). Last, ESCs are readily available because they can be obtained from fertility clinics (Pecorino, 2001).

There are several disadvantages associated with the use of ESCs. First, their use is ethically controversial. The ethical controversy is caused by the fact that in order to isolate human ESCs, human embryos must be destroyed (Condiac, 2002; Prentice, 2003). Second, ESCs have a high potential to get rejected by the recipient’s immune system. Third, they are often too complex to induce the appropriate cell type (Mackay-Sim, 2004; National Research Council & Institute of Medicine, 2002; Pecorino, 2001). Last, researchers have faced many difficulties getting the ESCs to start growing, to keep growing, and to differentiate within the laboratory cell culture (Commission on Life Sciences, 2002; Mackay-Sim, 2004; Pecorino, 2001; Prentice, 2003). Recently, a single cell extraction technique has been used that might, in the future, allow stem cell research without destroying embryos (Klimanskaya, Chung, Becker, Lu, & Lanza, 2006).

Adult stem cells. Although many researchers still believe that ESCs are the best hope for future treatments, many others point to the alternative of using adult stem cells (ASCs) (Mackay-Sim, 2004; Prentice, 2003). These stem cells are present in all adult tissues of the human body and certain types are able to regenerate throughout life (Commission on Life Sciences, 2002; Pecorino, 2001; Prentice, 2003). Researchers have known about ASCs and have used them for years in treatments for certain diseases such as bone marrow transplant for cancer and anemic patients (Commission on Life Sciences, 2002; Prentice, 2003). Although bone marrow cells have been studied the longest and seem to be one of the best sources of ASCs, research has shown that all tissues in the human body could be potential sources. Even the brain contains stem cells that can be extracted and “awakened” to form new brain cells and nerve cells (Mackay-Sim, 2004; Prentice, 2003). Interestingly enough, certain ASCs can “cross train” (transdifferentiation), meaning they can become tissues other than that of their specific origin. Although it may be difficult to obtain brain stem cells, it is fairly easy to obtain stem cells from bone marrow, fat, muscle, intestine, skin, or blood (Dazert et al., 2003; Prentice, 2003).

ASCs have been effective in treating certain diseased animal models and have recently been equally successful in treating human patients with diabetes, cerebral palsy, multiple sclerosis, stroke, immune deficiencies, osteoarthritis, tendonitis, coronary artery disease, dilative cardiomyopathy, macular degeneration, ALS (Lou Gehrig’s disease), traumatic brain injury, and numerous other diseases (The Institute for Cellular Medicine, 2006). Some advantages of ASCs are that they are already somewhat specialized, they pose less ethical concern than ESCs, the recipient’s immune system will not reject its own cells, and the cells are somewhat flexible to form other tissue types (Commission on Life Sciences, 2002; Mackay-Sim, 2004; Pecorino, 2001; Prentice, 2003). Disadvantages of ASCs are that they are difficult to acquire in large quantities, have a shorter life span when cultured, have a higher potential to carry genetic mutation or disease, and become defective during experimentation (Commission on Life Sciences, 2002; Mackay-Sim, 2004; Pecorino, 2001; Prentice, 2003).

Cell transplantation and the inner ear. Unlike sensory neurons in the vertebral olfactory system, which have the ability to continuously undergo neurogenesis and replacement, hair cell damage and death in the mammalian inner ear is considered to be permanent (Costanzo, 1985). Therefore, regeneration of hair cells from ESCs or ASCs is of great clinical interest. Although researchers have studied fish, amphibian, and avian regeneration, very little is known about the molecular mechanisms of the repair or regeneration processes in mammals, primarily humans (Dazert et al., 2003). Furthermore, it is currently assumed that during embryonic ear development, undifferentiated cells in the sensory epithelium (lining of the organ of Corti) differentiate into hair cells and their surrounding support cells (Dazert et al., 2003). Unfortunately, those stem cells have yet to be identified or isolated from the mammalian cochlea (Dazert et al., 2003; Holley, 2002). However, certain molecular switches and crucial protein factors for hair cell differentiation have been discovered and well studied (Dazert et al., 2003). This knowledge provides researchers with possible regulatory systems that...
they can apply in future research in attempts to generate new hair cells from ESCs or ASCs.

Although clinical application is limited, there has been one report of generation of sensory stem cells. Stefan Heller and fellow researchers at the Eaton-Peabody Laboratory at the Massachusetts Eye and Ear Infirmary have successfully isolated ASCs from the tissue of the mouse utricle. First, using ASCs, they developed a technique by which cells could be isolated and propagated in an artificial environment. The cells were then harvested in a medium supplemented with growth factors and nutrients for approximately 8 days (Keate, 2004; Li, Graham, Liu, & Heller, 2003; Watts & Corrales, 2004). The cells then began to form spheres, which were floating cloned colonies generated from a single stem cell and were made up primarily of progenitor cells that are capable of differentiating into different cell types (Keate, 2004). The researchers then grafted these cells into the ears of embryonic chickens. They then observed as the cells successfully integrated and began giving rise to new hair cells within the developing chicken ear (Keate, 2004; Li et al., 2003; Watts & Corrales, 2004).

The researchers then wanted to know if this transplant was possible using ESCs. They used the same growth conditions and began the process of differentiating the ESCs into aggregates called embryoid bodies (Keate, 2004; Li et al., 2003). When the cells formed the embryoid bodies, they were enriched with growth factors and later formed progenitor cells that expressed the genes, which indicated development in the inner ear. The cells were permitted to continue to differentiate until they expressed markers indicating hair cell growth (Keate, 2004; Li et al., 2003; Watts & Corrales, 2004). Therefore, cell transplant is possible, but clinical application in humans appears to be far in the future.

Drug Delivery Methods

Characteristics. Pharmaceutical technology is currently a source of scientific and medical importance, contributing to human health care. Until the 1970s, drug delivery focused primarily on fast-acting chemical compounds that are taken orally, in liquid or solid pill form, or through injections (Vogelson, 2001). Since then, there has been an increase in attempts to formulate drugs that are time released, meaning that the rate and length of release is controlled (Kumar, 2000). Once in the body, these polymers have also shown to improve absorption rates, lower body toxicity, and provide protection for the active agent against degradation (Vogelson, 2001).

These carriers containing the active agent must be introduced into the body by a delivery device (Biomedical Engineering Program, 2000). Currently, the most researched and applicable delivery devices for treating a multitude of various diseases and conditions are through oral delivery, injection, inhalation, transdermal, or implantable devices (Biomedical Engineering Program, 2000; Henry, 2000; Holley, 2002; Wilkoz & Bogner, 2003). There are numerous companies currently making advances in each method of delivery device. Each method has its own advantages and disadvantages, and finding the best choice of delivery system is often based on the needs of the individual (Henry, 2000).

Oral delivery involves simply swallowing a pill, and therefore is typically most preferred by patients (Henry, 2000; 3M, n.d.). Although it is convenient for the patient and easy to administer, oral delivery is limited to smaller chemical compounds (Henry, 2000). A primary reason for this difficulty is because the digestive system wants to metabolize large substances, thus making it harder for compounds found in pills to enter the bloodstream or other tissues within the body. Another disadvantage of oral delivery is that some treatments require the patient to take large numbers of pills, which may be a challenge for many patients (Henry, 2000).

Injection is another preferred method of drug delivery. Injection requires that drugs be administered with a needle into a specific region of the body. This method is problematic because some patients dislike the pain that accompanies the injection, the needle is unable to reach some target areas, and a medical professional may need to administer such treatments. However, drug companies have recently begun devising user-friendly injection devices. These gas-driven pumps can be worn on the back, chest, or abdomen, and deploy a needle with the push of a button. These needles are devised to deliver the drug at a constant rate until the entire reservoir is expended (Henry, 2000). Thus, the device offers increased convenience and compliance to the patient.
Another method of drug administration is pulmonary delivery. Pulmonary delivery involves administering therapeutic molecules into the body through the pathways of the lungs (Henry, 2000; 3M, n.d.). This method is noninvasive and involves rapid absorption of the active agent into the bloodstream (Henry, 2000; 3M, n.d.). The disadvantage of inhalation is that only small molecules can be delivered in such a manner, and the efficacy of the pulmonary device depends on the patient’s ability to use the device properly. Typically, the patient must be able to coordinate his or her breathing to the release of the inhalation pump. Companies are currently devising strategies to help the patient synchronize inhalation (Henry, 2000; 3M, n.d.).

Transdermal systems are another method of drug delivery in which the active agent is administered through the pores of the skin and enters the circulatory system. The advantages of this method are that the device is convenient, is easy to administer, has a consistent release rate, and allows steady flow of the drug into the body (3M, n.d.; Wilkosz & Bogner, 2003). However, transdermal delivery carries a risk that local irritation may arise at the site of the application site. Also, the skin will only allow certain molecules to diffuse into the pores due to its protective functions and low permeability. Furthermore, damage to the transdermal patch can result in an improper or even toxic rate of drug release into the body (Wilkosz & Bogner, 2003).

Last, implantable devices are currently being used to deliver pharmaceuticals (Biomedical Engineering Program, 2000). These devices are implanted through surgical techniques into a specific area of the body. Within the body, the device controls release of the active agent and attempts to create the desired effects. Therefore, implantable drug delivery devices are advantageous because they can reduce the possibility of under- or overdosing and the number of necessary administrations. Furthermore, this method improves patient compliance, and the active agents provide more localized effects. However, this method also has disadvantages because it involves a costly surgical procedure, which carries surgical risks and complications. Furthermore, the body may reject the foreign device or the polymers that are implantable (Biomedical Engineering Program, 2000).

“In any case, the purpose behind controlled drug delivery is to achieve more effective therapies while eliminating the potential of under or overdosing” (Brannon-Peppas, 1997). Other advantages are increased patient compliance, optimal use of drug, maintenance of drug level, and the need for fewer dosages or administrations (Brannon-Peppas, 1997; Henry, 2000; Henry, 2002; Vogelson, 2001). However, the potential disadvantages of drug delivery systems cannot be ignored. Drug delivery systems may potentially cause toxicity or noncompatibility of the body with the polymer or active agent used. They may result in undesirable side effects, discomfort, or by-products from the entry or degradation into the body system. If implantation surgery is necessary, there is the risk of infection and excess pain due to the invasiveness of the procedure. Last, drug delivery systems are more costly than traditional pharmaceutical drugs (Brannon-Peppas, 1997).

In conclusion, drug delivery systems could be applicable in the future for getting the needed active agents into the inner ear space and to the organ of Corti. There, the drug delivery system will release the active agent, which may then stimulate regrowth of old hair cells or growth of new hair cells, thus restoring hearing ability in individuals who suffer from sensory hearing loss.

**Drug delivery methods in the inner ear.** Most typically, the active agent is introduced directly through perfusion into the cochlear or vestibular channels, or by diffusion across the round window (Holley, 2002). The drug delivery concept is not entirely new, but using this approach to target diseased or dysfunctional regions in the inner ear is fairly recent. Therefore, little research has been published on this approach, primarily due to the lack of knowledge of the active agents that are needed to stimulate hair cell growth (Holley, 2002). Many laboratories and companies are first attempting to create the chemical molecules or compounds that would stimulate the growth. These specialists then hope to use various drug delivery systems to facilitate the entry of the pharmaceutical. Currently, there is published research on only three companies and one privately funded researcher that claim to be in the clinical testing stages in their efforts to regenerate or generate human cochlear hair cells.

Sound Pharmaceuticals (SPI) is a private biopharmaceutical company located in Seattle, WA (2002). This company is attempting to develop prescription drugs that would protect and treat hearing loss, especially noise-induced hearing loss (NIHL). In the field of regeneration, SPI is developing drugs that can potentially restore hearing. Currently, SPI is attempting to produce compounds that will provoke specific cell cycle proteins that cause new cell division. These dividing cells have the capacity to become replacement auditory hair cells. So far, they have shown promising results in animal models and have been recognized by the National Institute of Deafness and other Communication Disorders for their efforts in sensorineural regeneration (Lynch & Kil, 2005; SPI, 2002).

In addition, SPI has developed a drug that has been shown to be effective in protecting and restoring hair cells that will or have been subjected to loud noise exposure (Lynch & Kil, 2005; SPI, 2002). However, this drug is in its preliminary stages and can only work before or immediately following exposure. In addition, this drug does promises to slow down the process of hair cell damage for those individuals with sensory hearing loss (Lynch & Kil, 2005; SPI, 2002).

A similar company, American BioHealth Group, located in San Diego, CA, claims to have created an over-the-counter oral drug that can be taken to enhance the intrinsic defense mechanisms of the cochlea before an acoustic or toxic insult and to enhance the regenerative capabilities of the cochlea after acute injury but before permanent injury (American BioHealth Group, 2004). However, it has not shown the same capabilities for genetic or autoimmune disorders (American BioHealth Group, 2004). This drug is called the Hearing Pill and is protected under the United States Navy and the State University Patent #6,177,434 (American BioHealth Group, 2004). In addition, the
American BioHealth Group is currently attempting to develop more drug remedies that prevent and treat hearing loss resulting from noise or chemical exposure. DURECT Corporation (2005) is another company that is making promising strides in hair cell regeneration. This pioneer company in drug therapy treatments is located in Cupertino, CA, and has recently merged with the former company IntraEar. DURECT’s primary focus is to create effective delivery devices that will allow the necessary compounds to enter the inner ear space. Currently, the company has several similar delivery device models. Their leading model, the DUROS® delivery system, is a small titanium cylinder that allows a continuous release of drugs into a specific body region over a chosen period of time. This system gets implanted and delivers drugs through a catheter that targets the specific site (DURECT Corporation, 2005). This device has many benefits to potentially viable treatments of the inner ear space; therefore, it is important in the efforts to regenerate hair cells (DURECT Corporation, 2005).

Last, Gao (2005) has been granted numerous patents for treatments on inner hair cells. Gao’s most recent patent received approval on April 9, 2005, and included the following description:

Compositions, methods, and devices are provided for inducing or enhancing the growth, proliferation, regeneration of inner ear tissue, particularly inner hair cells. In addition, provided are compositions and methods for prophylactic or therapeutic treatment of a mammal afflicted with an inner ear disorder or condition, particularly for hearing impairments involving hair cell damage, loss, or degeneration, by administration of a therapeutically effective amount of IGF-1 or PFG-2, or their agonists, alone or in combination. (Gao, 2005)

Gao is funded by Genentech, a research company located in San Francisco, CA, whose “clinical trials are crucial to the exploration of new therapies that address significant unmet medical needs” (Gao, 2005; Genentech, 2005). Although Gao’s formulations are still in patent format, he hopes to make significant contributions to the regeneration of sensory hair cells using his drug delivery system.

Gao and the three companies described above have established Web sites and have indicated advances within their laboratories. Currently, there are no drugs that have been approved by the U.S. Food and Drug Administration that protect or restore hearing from the effects of noise, ototoxic drugs, or aging (American BioHealth Group, 2004). Therefore, the possibility of using drug delivery systems to generate or regenerate hair cells is tangible, but the clinical application in humans is not yet applicable.

CONCLUSION

Degeneration and death of sensory hair cells and their associated spiral ganglion result in more than 80% of the cases of individuals with hearing impairments (Holley, 2002; Willot, 1991). Mammals are unable to regenerate damaged or dead hair cells unlike the fish, shark, reptile, and avian species. However, researchers have been and are currently able to study these species that have spontaneous hair cell regeneration and use them as important models to understand the regeneration process within those species, as well as the structure and function of the mammalian auditory system. From past studies, researchers have gained valuable knowledge and have begun conducting studies on ways to grow new or repair damaged hair cells within the mammalian inner ear.

More recent research has focused on three primary approaches that are attempting to regenerate hair cells. These approaches are gene therapy, cell transplant, and drug delivery. Each approach appears to have advantages and disadvantages. Although none of these approaches is currently being used for clinical applications in humans, each approach is making gradual advances in hopes that someday, hair cell regeneration in the human ear will be possible and sensory hearing loss will be restored.

REFERENCES


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