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Current Situation and Progress of Pig Model in Otology Research

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Abstract

Pig models are widely used in otological research. The establishment of common pig and miniature pig animal models has opened up new fields in otological research and is also an ideal large otological animal model. This article introduces the applications, current status, progress, advantages, and issues of using pigs as otologic animal models. It summarizes current research on pigs in the fields of hearing disorders, otitis, vertigo, cochlea, gene editing, and tissue engineering, among other otologic and audiological areas. These models are valuable for translating basic medical science into clinical applications. Based on this, platforms have been established for studying deafness and vertigo, cochlear implantation experiments, stem cell and gene therapy, and tissue engineering. These serve as ideal experimental models for the prevention and treatment of ear diseases, pointing toward new directions. This will bolster the promotion and application of pig models in the fields of tissue engineering and gene editing in the future.

Keywords

Pig, Miniature Pig, Animal Model

1. Introduction

Animal models play a critical and innovative role in otological research, where ideal models are key to advancements in the field [1]. Excellent otologic animal models offer several advantages: they align with experimental needs in electrophysiology and anatomy; provide a favorable cost-benefit ratio; are easy to raise and manage; comply with ethical standards in empirical research; and are amenable to genetic engineering and editing.

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Despite their importance, otologic animal models currently face many disadvantages. Traditional rodent models, for example, exhibit significant differences from humans in genetics, size, lifespan, anatomy, and electrophysiology, limiting their utility in surgical and treatment contexts. Even when experimental results from these models can be translated to clinical applications, further validation using large animal models is often necessary [2].

Large animals, such as sheep, present their own challenges:

Limited temporal bone volume;

Substantial differences in mastoid and ossicular chain structure;

Divergent electrophysiological characteristics;

High costs;

Operational difficulties [3].

Dogs face issues like lengthy research durations, breeding and taming difficulties, aggressive nature, ethical concerns, and high costs [4]. Similarly, monkeys also pose ethical concerns, along with high costs, limited availability, and operational challenges [1] [5].

In contrast, pigs offer several benefits as otologic models [6]. They share a high genetic homology with humans, are easy to breed and manage, are cost-effective, and present fewer ethical issues. Their ear anatomy, electrophysiology, and pathophysiology closely resemble those of humans [7], making them an ideal model [8] [9]. Their extensive use in medical research underscores their emergence as a perfect [4], comprehensive model for otology.

Research on pig models encompasses both fundamental and applied studies, with the emphasis currently placed on fundamental research, which has yielded considerable advancements. The advancements of fundamental research cover areas such as the investigation of deafness mechanisms stem cell treatment, gene therapy, and viral transfection models [10] [11] [12] [13]. On the applied research front, the focus is predominantly on the utilization of pig models for otologic surgical procedures and instructional purposes in the medical teaching field [14] [15] [16].

The construction and development of pig models have expanded the methodologies and depth of research into ear diseases. They have consistently uncovered issues, progressively refining and addressing the shortcomings of previous studies, thereby aligning the findings more closely with human conditions. This alignment opens up the potential for these findings to be applied in clinical medical practices. This article reviews the current literature on pig models, detailing their applications, current status, progress, advantages, and challenges within the field of otology. It aims to equip researchers with the necessary references for selecting and establishing the most appropriate pig models, ultimately guiding future research efforts into ear diseases for more effective outcomes.

2. Types and Current Status of Pig Applications in Otological Research

For many years, pigs have served as valuable experimental animals in Otological

Research. Initially, common pigs, including farm and domestic varieties, were predominantly utilized for training in ear surgery operations and for educational purposes. In recent years, miniature pigs have been developed for research on the pathogenesis and treatment of otologic diseases. Based on the current state of research, the following conclusions are drawn.

2.1. Common Pigs Are Primarily Used for Training and Teaching in Ear Surgery

Common pigs include both non-living and living domestic and farm pigs. They are cost-effective and widely used for surgical training and education. Non-living pig ear models are utilized to teach the identification of temporal bone and ear anatomy while living pig models are used for ear surgery training and various ossicular prosthesis implantation training [17] [18] [19].

2.2. Miniature Pigs Are Primarily Used to Research Otologic Disease Pathogenesis and Treatment Methods

They are smaller than common pigs, more straightforward to breed, have better tolerance, are easier to catch and handle for surgery, and are used for studying otologic diseases and treatment methods. Although more expensive and less available than common pigs, miniature pigs are used primarily in studies of anatomy, electrophysiological characteristics, the mechanisms and treatment of deafness, auditory implants, and tissue engineering [1] [15] [20] [21] [22] [23] [24].

3. Advantages of Pigs as Otologic Animal Models

3.1. General Advantages of Pig Models

Pigs have a high homology with humans, are easy to breed and manage, are affordable, and present no ethical issues [25] [26] [27]. Taking the Chinese Bama miniature pig as an example [28]: (1) Genetically stable with good consistency: The polymorphism information content (PIC) and average heterozygosity are 0.3501 and 0.3881, respectively, indicating that the Guangxi Bama miniature pigs exhibit relatively low average PIC and heterozygosity ($P < 0.05$), demonstrating good genetic uniformity and stability. (2) High fecundity: The first litter averages 8.5 piglets, with subsequent litters averaging 10. Early sexual maturity: Female pigs reach their first estrus at 70 days of age with a body weight of 5 - 6 kg; they reach sexual maturity at 90 - 110 days of age with a body weight of 8 - 11 kg. Male pigs can begin breeding at approximately 75 days of age, have a body weight of about 7.5 kg, and can produce normal offspring. (3) Easy to breed and manage: During the growth period of Guangxi Bama miniature pigs, a daily diet with 12% protein, 12.97 MJ/kg of energy, containing 0.65% lysine, 0.65% calcium, and 0.32% available phosphorus is sufficient to meet their normal growth and development needs. (4) High homology with humans: Among 24 hematological parameters of the Bama miniature pig, 14 are similar to those in humans;

out of 44 blood biochemical parameters, 18 are similar to humans. Additionally, the shape of their organs and their proportionate weight to body weight are also very similar to humans. (5) Meets animal ethics requirements [29]: Based on regulatory toxicology studies, compared with dogs or non-human primates, maintaining miniature pigs at a high welfare standard is easier.

3.2. Pigs Have Ear Anatomical Structures Highly Similar to Humans

Scholars have compared the anatomy of the pig temporal bone, confirming the high similarity of pigs' eardrums, the Eustachian tube of the middle ear, ossicular chain, tympanic cavity, mastoid air cell system, inner ear's cochlea, vestibule, semicircular canals, facial acoustic nerve, lateral recess, and cochlear nucleus of the brainstem to those of humans [16] [30] [31] [32].

3.3. Pigs Are Similar to Humans in Developmental Biology, Audiology, Vestibular Function, and Electrophysiology

Studies have shown that the structure and development of pigs' hair cells (HC), cochlear development, stria vascularis, the organ of Corti, and spiral ganglion are similar to humans [14] [33]. Pigs have hearing from birth with thresholds at 25 - 30 dB SPL, auditory brainstem responses (ABR), and auditory conduction pathways highly similar to humans [34] [35]. Studies of miniature pigs' vestibular function confirmed that their neck extensor and masseter muscles induced by intense sound stimulation have consistent myogenic potential latency and thresholds [36]. Moreover, it is proposed that the masseter muscle is the optimal detection site for vestibular evoked myogenic potentials (VEMP).

4. Current Status of Pigs as Otologic Animal Models Domestically and Internationally

Based on the current fruitful outcomes, a summary of the application and research progress of the otologic pig model is presented.

4.1. Application of Miniature Pigs in Ear Surgery Operations and Teaching

André Gurr *et al.* were the first to propose using ordinary pig temporal bones as substitutes for human temporal bones in anatomical training and surgical procedures [17] [31]. This allows for the simulation of surgeries such as ossicular chain reconstruction, prosthesis techniques, and ear microsurgery for training and teaching purposes. They also suggested that pig temporal bones could serve as an immunological research model.

Mohamed Elsayed *et al.* suggested that pigs can be used as models for otological teaching and research [18]. They found that the pontocerebellar angle, internal auditory canal, and auditory facial nerve bundle are similar to those in humans, simulating surgical environments for the pontocerebellar angle and inner auditory canal. This can be beneficial for ear neurosurgery training and neurophysiological research.

Yang Shiming *et al*., through the dissection and comparison of pig temporal bones, proposed the instructional role of pig temporal bones as substitutes for human temporal bones [11]. They established a teaching model for simulating ear surgical operations training [12]. They also confirmed that in miniature pigs, the pre-auricular approach surgery is an ideal method for endo cochlear potential recording, and the retroauricular approach surgery is perfect for internal ear implantation [37]. This provides an experimental foundation for measuring inner ear data and treating inner ear diseases.

4.2. Application of Pigs in Deafness Mechanism Research, Deafness Models, and Treatment Research

J P Pracy *et al*. proposed that pigs are ideal animal models for studying the pathogenesis of otitis media through pig temporal bone dissection [30]. Ikarashi *et al*. used a pig model to investigate the relationship between mastoid pneumatization and otitis media, studying the pathogenesis and pathological changes in middle ear pressure [13]. Hou Zhaohui *et al*. confirmed that miniature pigs are ideal animal models for researching the Eustachian tube [7].

Chen Zhiting *et al*. exposed miniature pigs to pulsed spark noise, establishing the world's first noise-induced hearing loss animal model in miniature pigs [38]. They used mPEC-PLGA-BSA-FITC-NPs nanoparticles for inner ear delivery, confirming that this is an ideal animal model for treating noise-induced hearing loss, conducting biological interventions, and studying internal ear molecular delivery, providing a research foundation for large mammal auditory function intervention studies.

Du Yi *et al*. found that Rongchang albino pigs exhibited hearing loss and pigment loss similar to Waardenburg syndrome type 2A [7] [10] [20]. They confirmed MITF-M gene mutations during embryonic development, affecting cochlear hair cells, and established the WS pig model with collapsing cochlear hair cells [39] [40]. Hai Tang *et al*. induced mutations using N-ethyl-N-nitrosourea (ENU) chemical mutagenesis, utilizing the advantages of the CRISPR/Cas9 system, leading to amino acid changes in the MITF gene and establishing a novel WS2A pig model with $MITF^{+/L247S}$ mutation, replicating clinical symptoms and molecular pathology of WS2A [41] [42]. Xu Cong *et al*. established a KIT gene mutation WS2 lineage with congenital bilateral severe sensorineural hearing loss (SNHL) and pigment reduction through ENU chemical mutagenesis in miniature pigs [43]. They confirmed that the KIT gene is pathogenic for the WS2 type, similar to the MITF gene. These various WS2-type pig models provide new insights into the etiology of hearing loss and novel treatment approaches, offering methods and pathways for research on deafness treatments such as stem cell transplantation.

Hao Qingqing *et al*. obtained albino bama miniature swine mutants with deafness through ENU-induced mutagenesis, establishing Mondini malformation pig model genetic lineages and the Bama miniature pig audiology database

[44]. They clarified the microscopic structural characteristics of Mondini malformation and ruled out the MITF gene as a possible pathogenic gene for this model. This model will provide:

- Avenues for research in discovering pathogenic genes for Mondini malformation.
- Understanding the mechanism of deafness.
- Genetic therapy.

Zhang Xueru *et al*. studied the inner ear of miniature pigs by injecting ouabain solution at different concentrations into the round window membrane (RWM) of the internal ear unilaterally, attempting to establish an animal model of auditory neuropathy [45]. By analyzing the relationship between hearing changes and drug concentration in miniature pigs, it was found that ouabain solution causes damage to spiral ganglion cells, and the degree of damage is concentration-dependent. The effect on hearing threshold is significant, but the impact on hair cells is insignificant. This animal model will be an essential biological tool for research on the pathogenesis of hereditary deafness, clinical treatment, and drug screening.

Zhang Liyuan *et al*. measured the auditory brainstem response and morphological changes of cochlear hair cells before and after the unilateral injection of neomycin sulfate into the periosteum of the temporomandibular mastoid process in miniature pigs [46]. They observed high-frequency hearing loss in both ears of miniature pigs, with a significantly higher hearing threshold on the injection side than on the contralateral side. The ciliary structure of the outer hair cells on the injection side was more severely damaged than on the contralateral side of the cochlear basement membrane, indicating that retroauricular administration is an effective local administration method. This model is ideal for studying unilateral drug-induced hearing loss in diseases such as Meniere's disease and sudden idiopathic hearing loss, providing a foundation for research and treatment.

4.3. The Application of Miniature Pigs in Research Related to Auditory Implants

4.3.1. Cochlear Implantation

Guo Weiwei *et al*. used miniature pigs as a large animal model for cochlear implant (CI) applicability research [20] [47] [48]. They conducted CI simulations in miniature pigs, obtained hearing recovery data, and confirmed that miniature pigs are valuable CI research models. Based on this, a new species, the Rongchang pig CI animal model, was developed, along with a standardized CI surgical procedure. This allowed the study of hearing changes before and after CI, facilitating improvements and updates to cochlear implant electrodes and electronic devices and evaluating their effectiveness or safety. It laid the foundation for establishing a cochlear implantation platform for large mammals and its practical application in clinical patients.

Liu Qian *et al*. researched the grinding round window niche models in miniature pigs to study the electrophysiological and histomorphology of cochlea in miniature pigs after abrasion of round window niches in Cochlear implant surgery [22]. With the change of hair cell morphology between the two groups in different depths of electrode implantation and the change of ABR threshold before and after CI surgery, they confirmed that the microphonic potential thresholds of the cochlea at different depths increased and had smaller amplitudes. Additionally, the inner hair cells (IHCs) of the RWE group at the bottom of the grinding round window niche were significantly damaged, resulting in higher ABR hearing thresholds and more pronounced differences in the low-frequency region after surgery. Therefore, cochlear implantation without drilling the round window niche can preserve some level of hearing and IHC function. This research supports the development of cochlear implant surgical techniques and devices without the grinding round window niche.

Erdem Yildiz *et al.* used domestic and miniature pigs as CI models for applicability research, obtaining data on postoperative trauma and partial hearing recovery [15] [23]. They provided data on the health changes in the cochlea of large animals following CI. Based on this, they used piglet cochlear cannulation as a pharmacokinetic model for cochlear implants and conducted controlled cochlear delivery studies using fluorescein isothiocyanate-dextran (FITC-d). They measured increased FITC-d concentrations at the top of the cochlea and total concentrations in perilymphatic samples, with a more even distribution after the concentration decrease. This method highlights the safety of cochlear canal inner ear delivery. It outlines the pharmacokinetic basis for treating the inner ear with otoprotective compounds.

4.3.2. Auditory Brainstem Implantation

H. J. YI *et al.* simulated and validated the surgical procedure of artificial auditory brainstem implantation (ABI) in miniature pigs via the translabyrinthine route [32]. This approach provided the optimal pathway and visual anatomical structures for accessing the cochlear nucleus and internal auditory canal, laying the foundation for clinical applications of ABI in treating cochlear lesions.

4.4. Application of Miniature Pigs in Stem Cell Therapy, Gene Therapy, and Viral Transfection Models

4.4.1. Stem Cell Therapy

Ma Yueying *et al.* used a lumbar subarachnoid puncture-cerebrospinal fluid route for stem cell transplantation in a miniature pig model to treat spiral ganglion cell and auditory nerve lesions [49]. They successfully migrated stem cells to the cochlea, which impacted ABR in hearing-impaired Rongchang pigs. This model applies to sensorineural hearing loss (SNHL), hair cells, and auditory nerve lesions. It holds significant importance for studying the pathogenesis of WS2A syndrome and stem cell transplantation approaches.

Xu Liangwei *et al.* tracked and treated noise-induced hearing loss (NIHL) pig models using superparamagnetic iron oxide (SPIO)-labeled human umbilical cord mesenchymal stem cells (UC-MSCs) [50]. They confirmed that UC-MSCs transplantation is an effective method for treating SNHL. This method has broad prospects in applying stem cell therapy and tracking transplanted UC-MSCs, providing a foundation for researching damaged hair cells and stem cells' targets and signaling pathways.

Wang Cuicui *et al.* conducted induced pluripotent stem cell (iPSCs) transplantation research using Rongchang pigs with Mitf-M gene mutations [16]. They demonstrated that transplanted iPSCs induced differentiation of inner hair cells (IHCs) and supporting cells, forming tight and gap junctions in vitro and in vivo. This contributes to research and development in stem cell therapy for hearing loss.

4.4.2. Gene Therapy and Viral Transfection Models

Shi Xunbei *et al.* used the round window membrane (RWM) method to transfect miniature pig inner hair cells with adeno-associated virus (AAV1-GFP), confirming the effectiveness and safety of cochlear transfection [51]. This method allows for rapid access to the RWM, protects the ossicular chain and eardrum structures, and does not affect hearing and balance abilities. This model research provides reference and assurance for hearing loss gene therapy and the clinical application of AAV1.

Ji Xiaojun *et al.* used the AAV1-CMV-GFP vector to deliver genes to the inner ear of miniature pigs through three methods: RWM, horizontal semicircular canal, and posterior semicircular canal (PSC) [24]. They evaluated the differences in ABR thresholds among the three methods. They found that PSC had the most negligible impact on auditory function, the widest reagent distribution, easy anatomical recognition, and minimal temporal bone damage, leading to improved gene expression and function. This model provides an essential experimental basis for inner ear gene therapy development.

4.5. 3D Printing Technology and Tissue Engineering in Otological Research

David A. Zopf *et al.* utilized 3D printing technology to create absorbable poly-ε-caprolactone scaffolds for bioengineering [21]. These scaffolds were implanted into the ears and noses of pig models. In the case of auricular scaffolds, chondrogenic growth factors were seeded within hyaluronic acid/collagen hydrogels, and the scaffolds were cultured ex vivo, resulting in the development of nasal and auricular scaffolds that support cartilage regeneration. This work laid the foundation for 3D printing technology and tissue engineering in the field of otolaryngology, providing valuable insights for future ear tissue engineering and reconstruction applications.

5. Challenges and Issues Associated with Using Pig Models in Otological Research

There are still numerous issues in animal model research, and pigs, which are considered an ideal model for otolaryngology, also have disadvantages and limitations. Minimal pigs face various challenges in ear research, which are summarized below.

5.1. Comparison with Traditional Ear Models [1] [4] [52]

Poor cost-effectiveness in experiments, demanding, and relatively high maintenance conditions. Longer experimental cycles and slower turnover. We have limited sample sizes due to economic costs and environmental constraints in experimental designs.

5.2. Anatomical Differences from Humans [31] [32]

Compared to humans, there are morphological differences in the temporal bone, external ear canal, mastoid position, and cochlear size of pigs. Narrow and tortuous external ear canals in miniature pigs, with the posterior wall of the tympanic cavity being the semicircular canal and the lower wall poorly aerated, resembling a plate-like mastoid similar to humans. The pig cochlea has 3.5 turns, more than the human cochlea. These differences impose limitations on ear anatomy and surgical training.

5.3. Auditory Differences from Humans [14]

There are subtle differences in the waveform of auditory brainstem response (ABR) between pigs and humans: pig ABR typically shows a fusion of III and IV waves. In contrast, human ABR offers a fusion of IV and V waves. Differences in the auditory threshold, sensitive hearing frequencies, and threshold ranges: Pig auditory threshold is generally between 25 - 30 dB SPL, slightly higher than humans. A pig's threshold range can reach up to 48 kHz, exceeding the human range of 20 - 20 kHz. The most sensitive hearing frequency for humans is 1 kHz, while for pigs, it is 2 kHz. Hence, research results from miniature pigs may only partially apply to humans when studying certain hearing disorders.

These limitations in miniature pig models impact the reliability and representativeness of research outcomes and restrict the extensive application of pigs as large animal models, which is a common challenge for other large animal models. Therefore, when using miniature pigs for experiments, it is essential to consider these limitations and take necessary measures to ensure the accuracy and reliability of research results.

Based on scholars' research, it is believed that pig models have good application prospects, but they are not perfect. There is a need to establish a complete system of otology animal models, progressing step by step and adapting to local conditions. The following suggestions are proposed for the future development of pig models: (1) Strengthen the development and protection of miniature pig models, perfect the supply system, and standardize breeding and management. (2) Establish and perfect the standards for experimental miniature pig models, thereby promoting the development of large animal models in otology and advancing the research into ear diseases; (3) Utilize emerging technologies such as gene editing, somatic cell nuclear transfer, 3D printing, and tissue engineering to establish more miniature pig disease models that meet the requirements of otology research. Experience is summarized based on existing models. Pig models can be divided into 3 tiers: (1) The first tier involves using live and non-live ordinary pigs for otology anatomy teaching and surgical operation training; (2) The second tier involves using miniature pigs for research on the mechanisms of deafness, deafness models and treatment, and the application of auditory implants; (3) The third tier involves using miniature pigs for applications in stem cell therapy, gene therapy, viral transfection models, 3D printing technology, and tissue engineering research. For example, medical colleges and surgical skill centers use the first-tier pig models for learning, ear disease research institutes, deafness prevention centers, and auditory implant R&D centers use the second-tier pig models, and ear disease research institutes, deafness prevention centers, and otology tissue engineering centers use the third-tier models. The three tiers promote each other, with the third tier serving as the main direction for future development, vigorously exploring and perfecting the research fields of gene editing and tissue engineering reconstruction. This may represent an epic innovation and guide in the research and treatment of ear diseases.

In summary, pig models have pioneered a new field in otological animal models, mainly used in otological surgical operations and teaching, research related to the mechanisms and treatment of deafness, studies on auditory implants, stem cell therapy, gene therapy, and viral transfection models, and tissue engineering research. They have facilitated the transition from basic medical science to clinical medical applications. Based on this, various platforms for research on the mechanisms of deafness and vertigo, experimental platforms for cochlear implantation, stem cell and gene therapy, and tissue engineering reconstruction have been established. These provide the ideal experimental models for the prevention and treatment of ear diseases and point towards the latest directions, with the hope of strengthening the promotion and application of pig models in tissue engineering reconstruction and gene editing in the future.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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