

REVIEW ARTICLE

Inner ear organoids: From three-dimensional bioprinting to vascularization

Fuzheng Li¹, Kexin Yang¹, Mohsen Asadnia², Jianxia Chen³, and Guozhen Liu^{1,4*}

¹Integrated Devices and Intelligent Diagnosis (ID2) Laboratory, CUHK(SZ)-Boyalife Joint Laboratory for Regenerative Medicine Engineering, School of Medicine, The Chinese University of Hong Kong, Shenzhen, Guangdong, China

²School of Engineering, Macquarie University, Sydney, New South Wales, Australia

³Clinical Laboratory, Longgang Central Hospital of Shenzhen, Shenzhen, Guangdong, China

⁴Guangdong Basic Research Center of Excellence for Aggregate Science, School of Science and Engineering, Shenzhen Institute of Aggregate Science and Technology, The Chinese University of Hong Kong, Shenzhen, Guangdong, China

*Corresponding author: Guozhen Liu (liuguozhen@cuhk.edu.cn)

Abstract

Citation: Li F, Yang K, Asadnia M, Chen J, Liu G. Inner ear organoids: From three-dimensional bioprinting to vascularization. *Organoid Res.* 2026;2(1):025010041.
doi: 10.36922/OR025010041

Received: December 31, 2025

Revised: February 26, 2026

Accepted: February 26, 2026

Published online: March 12, 2026

Copyright: © 2026 Author(s). This is an Open-Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, which provided that the original work is properly cited.

Publisher's Note: AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

The inner ear is essential for auditory perception and balance, yet damage to cochlear hair cells remains a major cause of irreversible hearing loss worldwide. As the number of individuals affected by sensorineural deafness continues to rise, there is a growing need for deeper pathophysiological insight and the development of effective therapies. Inner ear organoids, which recapitulate key aspects of the in vivo inner ear microenvironment, have emerged as powerful platforms for disease modeling, high-throughput drug screening, and regenerative medicine. This review summarizes recent progress in the development of inner ear organoids, with a focus on strategies to improve their structural complexity and functional maturity. In particular, we highlight innovations in vascularization and three-dimensional bioprinting that are advancing organoid scalability and integration potential. Meanwhile, commonly used biomaterials in three-dimensional bioprinting for inner ear organoids are systematically compared. Finally, we discuss current challenges and future directions for translating these technologies into clinical applications.

Keywords: Inner ear; Organoids; Vascularization; Three-dimensional bioprinting; Biomaterials; Hair cell regeneration

1. Introduction

The inner ear is a delicate organ with multiple cell types and complex tissue organization located deep inside the temporal bone. It contains inner and outer layers of hair cells, which perform mechanoelectrical transduction through deflection of stereociliary bundles, thereby enabling auditory perception.¹ Hair cells are vulnerable to a variety of ototoxic factors, including loud noise, aging, genetic defects, and ototoxic drugs.² Once hair cells are damaged, the inner ear loses its ability to transduce auditory signals to the cerebral cortex, causing hearing loss.

Hearing loss due to hair cell death has become a growing health burden globally.³ The 2025 World Health Organization report indicates that rehabilitative care is essential for 430 million individuals globally (representing >5% of the world's population) who are living with disabling hearing loss.³ Current treatment options for hearing loss are mostly based on medical devices, such as hearing aids and cochlear implants, which may cause symptoms such as ear canal pressure, inflammation, and difficulty concentrating on auditory stimuli in some patients. Research focused on gene therapies for hearing loss and targeted drugs that stimulate hair cell regeneration or protect hair cells usually

employs animal models. However, animal models exhibit inherent limitations in replicating human physiological conditions. Primarily, genetic background disparities between species may prevent human deafness-associated mutations introduced into murine genomes from generating sufficient phenotypic manifestations of hearing loss.⁴ Secondly, the significantly higher permeability of the blood–labyrinth barrier (BLB) in murine models compared to humans substantially restricts their utility for drug screening applications.⁵

Since Koehler *et al.*⁶ successfully generated inner ear organoids with mechanosensitive hair cells bearing stereociliary bundles and functional synaptic connectivity from human pluripotent stem cells (hPSCs) *in vitro* in 2017, inner ear organoids have garnered increasing attention in the scientific community. These organoids, which emulate the cellular complexity and genetic fidelity of native human tissue, show distinct advantages over traditional animal models and serve as platforms for discovering regenerative mechanisms.⁷ Research demonstrates that inner ear organoids exhibit superior accuracy in predicting drug responses and enhanced efficiency in drug screening compared to murine models.⁸ Single-cell RNA sequencing confirms that these organoids closely recapitulate embryonic developmental programs with high genomic and transcriptomic fidelity. Furthermore, the generation of otic vesicles within organoids developing in a quasi-*in vivo* manner enables detailed investigation of key epithelial and mesenchymal cell populations affected by genetic mutations.⁹

However, current organoid systems remain constrained by incomplete biomimicry. The absence of spatially organized three-dimensional (3D) architecture and dynamic extracellular matrix (ECM) gradients limits their utility in modeling complex pathologies or screening therapeutics. While recent reviews have documented progress in organoid generation and applications, few have systematically addressed vascularization.^{1,10,11}

Lou *et al.*¹⁰ reviewed inner ear organoid applications in disease modeling, drug screening, and hair cell regeneration, with limited discussion on vascularization strategies and 3D bioprinting technologies. Although Moeinvaziri *et al.*¹² examined vascularization, their review lacked a systematic evaluation of strategy-specific parameters and inner ear-specific requirements, such as BLB reconstruction. Pianigiani and Roccio¹³ discussed the strengths and limitations of hPSC-derived inner ear organoids, focusing on cell composition variability, fetal-like maturity, and assay readout challenges; however, their discussion did not extend to bioengineering solutions such as vascularization, scaffold-based mechanotransduction, or bioprinting-enabled tissue architecture reconstruction.

This review explores engineering challenges—from 3D bioprinting to vascularization—associated with inner ear organoids that currently impede functional maturation and clinical translation (Figure 1). Firstly, the evolution of inner ear organoid development from early two-dimensional (2D) hair cell-like cultures to contemporary 3D multicellular systems is examined, establishing the biological foundation and identifying critical maturation bottlenecks. Subsequently, vascularization—a prerequisite for scaling functional constructs—is discussed through the lens of inner ear-specific requirements, particularly the tripartite cellular architecture of the stria vascularis and BLB reconstruction.

Unlike generalized vascularization reviews, we systematically evaluate hypoxia, growth factor delivery, co-culture, and scaffold-based approaches according to criteria unique to the inner ear microenvironment, including ion transport capacity and endocochlear potential maintenance. Moreover, 3D bioprinting technologies and biomaterial selection are analyzed to elucidate how scaffold mechanical properties influence cochlear progenitor cell fate via mechanotransduction pathways. Finally, the review discusses translational applications in drug screening, disease modeling, and regenerative medicine, followed by current challenges and future perspectives, while highlighting the emerging convergence of vascularization engineering and 3D bioprinting as a promising strategy for achieving clinically relevant inner ear organoids.

2. Background on the inner ear

2.1. Development of the inner ear

The vertebrate inner ear is a complex structure derived from a thickened ectodermal region termed the otic placode.^{14,15} During the third week of embryonic development, the otic placode invaginates to form a 3D spherical structure called the otocyst, or otic vesicle.¹⁶ The ventral otic vesicle forms the cochlea and saccule, whereas the dorsal otic vesicle forms the remainder of the vestibular structure. Vacuoles appear within the ventral otic vesicle and develop into spaces, which differentiate into scala tympani, scala media (or cochlear duct), and scala vestibuli.^{17–19} Scala media is filled with endolymph, which possesses a high concentration of potassium, while the scala vestibuli and scala tympani are perfused with perilymph.²⁰ The epithelial cells of the cochlear duct differentiate into specialized sensory structures, including inner and outer hair cells and supporting cells.²¹

2.2. Inner ear organoid development from 2D culture to 3D multicellular organization

Significant advancements in the creation of inner ear organoids have been made over the past 20 years. The organoids' initial 2D planar architecture has given way to

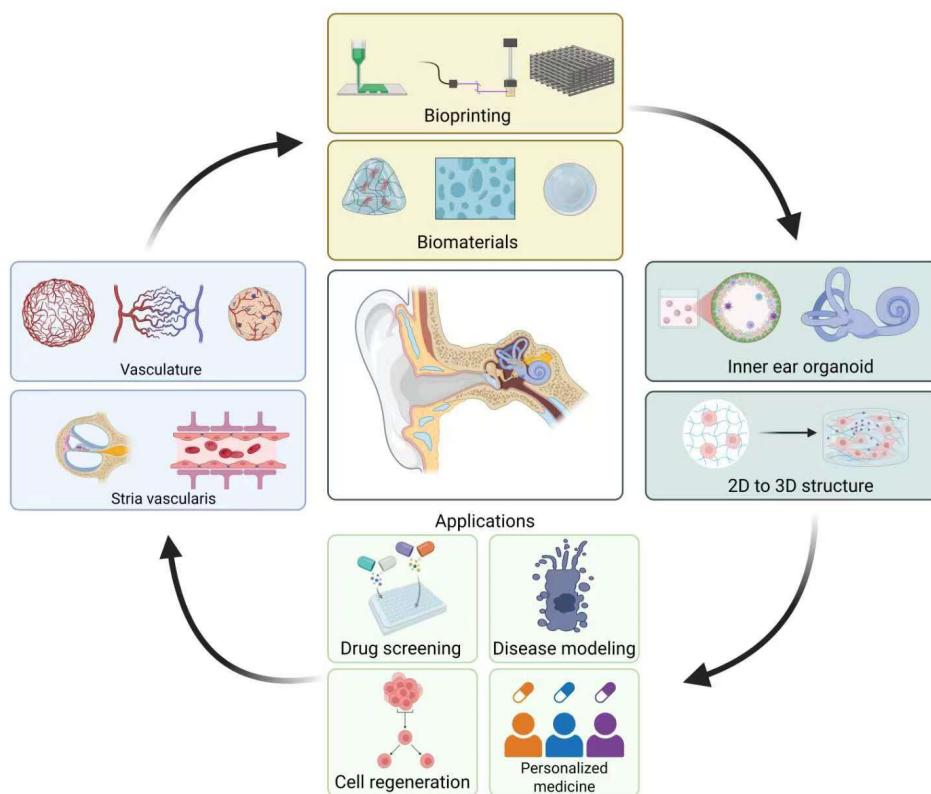


Figure 1. Overview of inner ear organoid construction. Created using BioRender. Li, F. (2026) <https://BioRender.com/gtvj8ch>.
Abbreviations: 2D: Two-dimensional; 3D: Three-dimensional.

3D structures, and cell culture techniques have progressed from monoculture systems to multicellular co-culture strategies. This section summarizes the historical development of inner ear organoids, along with potential future directions for research.

Li *et al.*²² found that inner ear progenitor cells and mouse embryonic stem cells (mESCs) could both be transformed into cells that resemble hair cells. This paradigm was extended by Oshima *et al.*²³ by isolating multipotent stem cells from the spiral ganglia, vestibular organs, and cochlea of mice. They accomplished the simultaneous differentiation of hair cells, neurons, and glial cells by using reverse transcription polymerase chain reaction-based lineage tracing and targeted growth factor supplementation (e.g., fibroblast growth factor [FGF]-2, insulin-like growth factor [IGF]-1). Using mESCs and induced pluripotent stem cells (iPSCs), Oshima *et al.*²⁴ enhanced the specificity of stem cell differentiation toward ectoderm by inhibiting the Wnt and transforming growth factor- β (TGF β) pathways, and subsequently induced the formation of *Pax2*⁺ otic progenitor cells by adding IGF-1 to activate the IGF and basic FGF pathways, thereby obtaining hair cell-like cells expressing marker molecules such as espin and transmembrane channel-like protein 1 for the

first time. When mechanical stimulation was applied, these cells could generate transduction currents that closely resembled those of mature hair cells.²⁴

By growing and developing into cells that resemble hair cells *in vitro*, studies showed that *Lgr5*⁺ progenitor cells, which were initially identified as markers of intestinal stem cells, are susceptible to Wnt signaling.²⁵⁻²⁷ These differentiated cells produced stereociliary bundles enriched with F-actin and espin, which are structural characteristics of functional mechanosensory hair cells, in addition to expressing classical hair cell markers, including *Myo7a* (encodes myosin VIIA). Expanding on this finding, Shi *et al.*²⁸ further demonstrated that Wnt stimulation might achieve limited proliferation *in vivo* by temporarily re-entering *Lgr5*⁺ cells in the post-mitotic cochlear sensory epithelium of adult mice into the cell cycle. A possible regenerative approach to age-related or noise-induced hearing loss was suggested by this discovery, which also challenged the long-held belief that mammals permanently lose their hair cells.

Prior to 2013, research was limited by the types of stem cells and culture methods available, which prevented the formation of organoids with multicellular structures. In

2013, Koehler *et al.*²⁹ pioneered the generation of inner ear organoids from mESCs within a 3D culture system, achieving the simultaneous differentiation of sensory neurons and hair cells. Bone morphogenetic protein (BMP)-4 activation directed the cell aggregates toward the non-neural ectoderm lineage before the BMP signal was attenuated using the inhibitor LDN193189, concurrent with the introduction of FGF2 to promote otic placode specification. Finally, the addition of CHIR99021 activated the Wnt pathway, driving the synchronous differentiation of hair cells and neurons, as well as synapse formation. This precisely timed sequence of BMP activation followed by inhibition closely recapitulated the *in vivo* signaling dynamics during otic development.

Building upon this foundation, McLean *et al.*³⁰ discovered that the combined administration of the histone deacetylase inhibitor valproic acid, which antagonizes Notch-mediated differentiation signals, and the glycogen synthase kinase-3 β inhibitor CHIR99021 resulted in a five-fold expansion of *Lgr5*⁺ cochlear supporting cells. Furthermore, upon withdrawal of these stimuli over 14 days, 60% of these cells differentiated into *Myo7a*⁺ hair cells.³⁰

In 2017, Koehler *et al.*⁶ first reported a method for generating functional inner ear organoids using human embryonic stem cells (hESCs). During cultivation, the Matrigel-embedded cell aggregates were treated with CHIR99021 to activate the Wnt pathway and promote target gene expression, with the aggregates exhibiting epithelial protrusions within 12–16 days. Through additional culture, a large number of epithelial structures resembling otic plates and otocysts were produced. Some otocysts had sensory epithelium with specialized features that resembled hair cells and displayed high F-actin apical junctions. Following 40–60 days of incubation, vesicles exhibiting complex multicavity morphology became visible through the aggregate surface, with cells expressing multiple hair cell markers, including *MYO7A*, *PCP4*, and *SOX2*. The study also showed that human inner ear organoids created a sensory neuronal network between sensory neurons and hair cells, although it remains unknown whether these organoids possess electrophysiological functionality.

The method of distinguishing inner ear organoids from hair cell-like cells was examined by Roccio *et al.*³¹ in 2018. The research involved seeding *EPCAM*⁺ cells derived from the human cochlear duct onto a culture medium, supplemented with Matrigel to form epithelial organoids. These organoids were then co-cultured with *EPCAM*⁺ cells and differentiated into hair cell-like cells expressing markers such as *MYO7A* and *SOX2* under the influence of CHIR99021 and γ -secretase inhibitors.

In 2023, Xia *et al.*³² successfully cultured large-scale inner

ear organoids with physiological synapses in an optimized co-culture matrix. The researchers improved the culture protocol by co-culturing cochlear hair cells from mice and spiral ganglion neurons (SGNs) at a distance of 100–300 μ m. Within 14 days, *TUJ1*⁺ neurites expanded directionally toward hair cells, creating glutamatergic synapses that were functionally capable of synaptic transmission, as demonstrated by L-glutamate-evoked Ca²⁺ transients. This work created a scalable platform for sensorineural circuit modeling, while also demonstrating physiological neuron–epithelium crosstalk.

In the same year, a significant breakthrough was achieved when Moore *et al.*³³ developed a protocol for generating high-fidelity cochlear organoids from hPSCs. Unlike earlier vestibular-biased protocols, this approach specifically directed differentiation toward cochlear fates through temporally controlled modulation of FGF, BMP, and Wnt signaling. The resulting organoids contained hair cells with tonotopically organized stereocilia bundles and functional mechanotransduction channels, representing the closest recapitulation of the human cochlea achieved to date. Critically, this work demonstrated that cochlear and vestibular organoids require distinct signaling trajectories for disease modeling of frequency-specific hearing loss.

3. Inner ear organoid: From medium nutrient to vascularization

Although current inner ear organoids have developed functional hair cells and neural synapses and are already being utilized in experimental applications such as drug screening, they still face limitations, including restricted scale, the absence of microvascular networks for nutrient supply, insufficient cellular diversity, and an inability to form complex spiral architectures.^{9,34} Unlike previous reviews that have addressed vascularization strategies in a generalized context,¹² this review uniquely centers on the engineering challenges posed by the tripartite cellular architecture of the BLB.

We propose an integrative framework encompassing “vascularization–mechanotransduction–ion homeostasis,” specifically tailored to the inner ear microenvironment. Critically, vascularization of inner ear organoids fundamentally differs from that of brain organoids. While cerebral vascular networks primarily serve nutrient delivery and waste removal,³⁵ the stria vascularis functions as an ion-transporting epithelium that actively maintains the endocochlear potential through K⁺ cycling via Na⁺/K⁺-ATPase and Kir4.1 channels.³⁶ This functional specificity mandates that engineered microvascular networks not only recapitulate the marginal–intermediate–basal cell layering but also express tight junction proteins and ion channels essential for endolymph homeostasis. By systematically evaluating diverse vascularization approaches against these

inner ear-specific criteria, this review provides a novel perspective for advancing the functional maturation of inner ear organoids beyond current limitations.

Given that inner ear organoid vascularization and bioprinting represent emerging fields with limited direct evidence, this review strategically incorporates findings from more extensively characterized organoid systems. The selection of studies for this review was guided by the following criteria: the research either shared a common developmental origin with inner ear organoids (brain organoids derived from the ectoderm), addressed analogous barrier formation requirements, followed transferable biophysical principles (the effects of fluid shear stress and matrix stiffness on cell differentiation), or involved conserved molecular regulatory mechanisms.

3.1. Vascularization in inner ear organoids

Different from cerebral organoids, the stria vascularis of the inner ear represents the sole example of a vascularized secretory epithelium in mammals, wherein the vasculature actively participates in generating and maintaining the endocochlear potential gradient essential for mechanotransduction in hair cells.³⁷ This functional specificity necessitates an integrative conceptual framework encompassing three interdependent pillars: vascularization, mechanotransduction, and ion homeostasis.

As illustrated in Figure 2, this section discusses the unique physiological and anatomical architecture of the

stria vascularis to establish the engineering criteria that vascularization strategies must fulfill, systematically evaluates the translational prospects of each approach against these inner ear-specific benchmarks, and ultimately proposes an integrated framework tailored for constructing functionally mature vascularized inner ear organoids.

The vascularization component must recapitulate the tripartite cellular architecture of the stria vascularis, comprising marginal cells facing the endolymph, intermediate cells of neural crest origin, and basal cells forming the BLB.³⁷ Unlike conventional endothelial networks, this configuration requires coordinated expression of ion transport machinery collectively mediating K^+ recycling from perilymph to endolymph.³⁸ Additionally, mechanotransduction signals derived from the ECM critically regulate both vascular and sensory epithelium development. Emerging evidence demonstrates that matrix stiffness modulates cochlear progenitor cell fate through integrin $\alpha 3$ /Yes-associated protein-mediated mechanosensing during expansion, and Ca^{2+} -dependent extracellular signal-regulated protein kinase/Krüppel-like factor 2 activation during differentiation.³⁴ These mechanobiological cues are intrinsically linked to vascular function, as the mechanical properties of bioengineered scaffolds influence endothelial cell proliferation, tight junction formation, and barrier integrity.³⁹ Moreover, ion homeostasis represents the ultimate functional readout, as the endocochlear potential depends on the coordinated

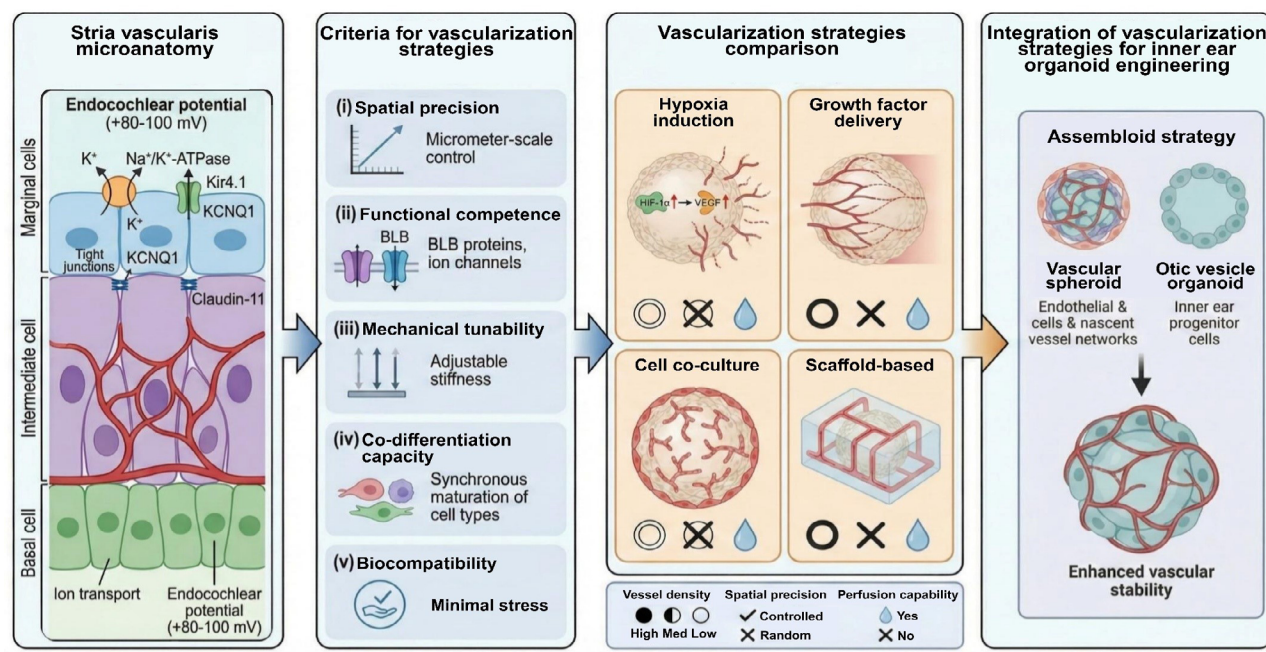


Figure 2. Tripartite cellular organization of the stria vascularis and the vascularization-mechanotransduction-ion homeostasis framework. Created using BioRender. Estella, A. (2026) <https://BioRender.com/am9kxl3>.

activity of multiple ion channels and the integrity of tight junction complexes (e.g., claudin-5, claudin-11, occludin) that prevent ionic equilibration between vascular and endolymphatic compartments.

Based on the above analysis, constructing a functionally mature vascular network for inner ear organoids necessitates vascularization strategies that simultaneously meet key technical requirements. First, they must possess extremely high spatial precision to regulate vessel spacing and branching density at the micrometer scale, thereby reconstructing the unique trilaminar architecture comprising marginal, intermediate, and basal cells. Physiological data indicate that native strial capillaries

range from 5 to 20 μm in diameter with a length density of 360–484 mm/mm^3 ,⁴⁰ providing critical morphological parameters for engineered constructs.

Second, the engineered vasculature must exhibit full functional competence, supporting the proper expression of BLB-specific tight junction proteins and key ion channels essential for K^+ transport. Third, scaffold materials must offer mechanical tunability, enabling dynamic modulation of matrix stiffness to guide precise cell fate decisions via mechanotransduction pathways during distinct differentiation stages. Fourth, the culture system should possess co-differentiation capacity, allowing for the simultaneous induction and maturation of endothelial cells,

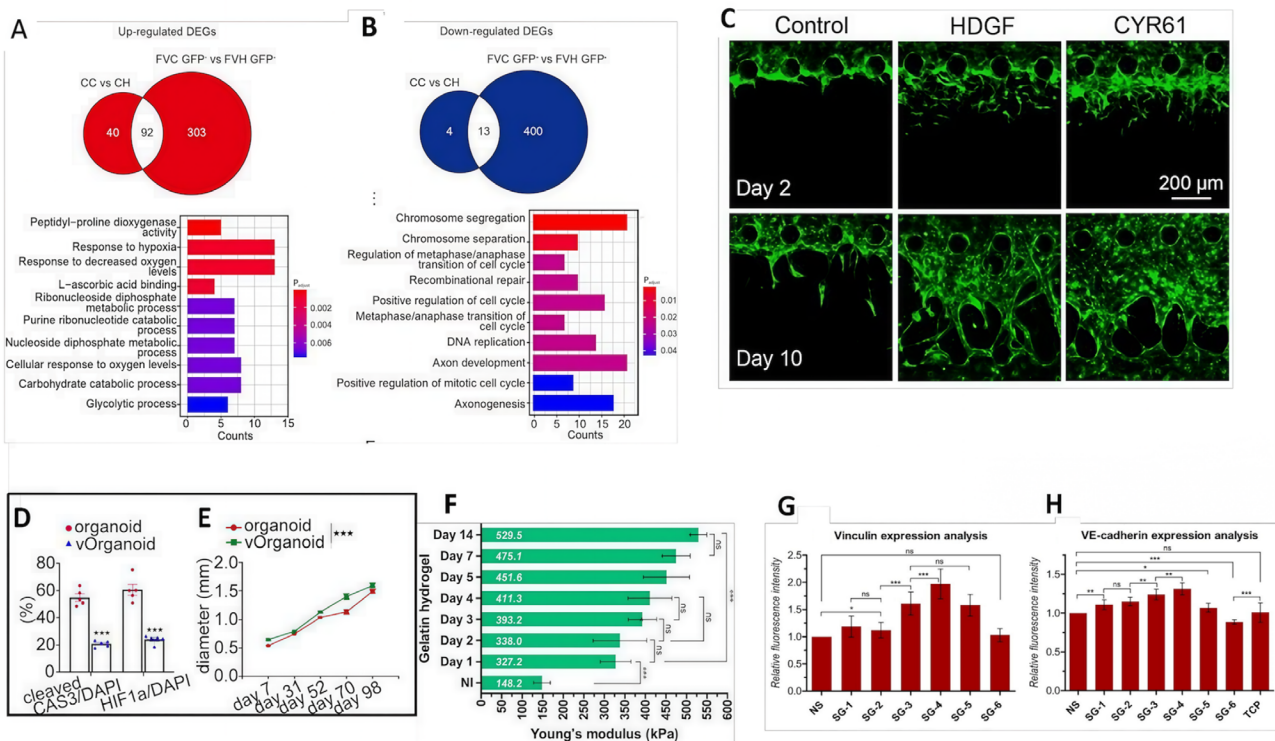


Figure 3. Transcriptomic and functional analysis of vascularization strategies in inner ear organoid systems. (A) Venn diagram of upregulated DEGs in Cors and FVCors after hypoxia, with Gene Ontology analysis of the 343 DEGs in the Venn diagram (adjusted $p < 0.05$). Reproduced with permission from Li *et al.*⁴¹ Copyright © 2025, Springer Nature. (B) Venn diagram of downregulated DEGs in Cors and FVCors after hypoxia, with Gene Ontology analysis of the 404 DEGs in the Venn diagram (adjusted $p < 0.05$). Reproduced with permission from Li *et al.*⁴¹ Copyright © 2025, Springer Nature. (C) Angiogenic sprouts (shown in green) at days 2 and 10 of culture in EGM2 only (control), EGM2 with HDGF, and EGM2 with CYR61 (scale bar: 200 μm). Reproduced with permission from Shaji *et al.*⁴² Copyright © 2024, Royal Society of Chemistry. (D) Quantification of cleaved CAS3 and HIF-1 α cells as a percentage of total cells (DAPI) in control organoids and vOrganoids at day 115, respectively. Reproduced with permission from Shi *et al.*⁴³ Copyright © 2020, Public Library of Science. (E) Diameters of organoids and vOrganoids generated from H9 at days 7, 31, 52, 70, and 98, respectively. Reproduced with permission from Shi *et al.*⁴³ Copyright © 2020, Public Library of Science. (F) Young's modulus of gelatin hydrogels before and after 14 days of incubation. Reproduced with permission from Salehi *et al.*⁴⁴ Copyright © 2025, Taylor & Francis. (G) Vinculin expression in endothelial cells on control (non-structured scaffold) and surface-structured hydrogel scaffolds. Reproduced with permission from Salehi *et al.*⁴⁴ Copyright © 2025, Taylor & Francis. (H) VE-cadherin expression in endothelial cells on control (non-structured scaffold) and surface-structured hydrogel scaffolds. Reproduced with permission from Salehi *et al.*⁴⁴ Copyright © 2025, Taylor & Francis. Notes: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ indicate statistical significance; whereas “ns” indicates not significance.

Abbreviations: CAS3: Caspase-3; CC: Cors control; CH: Cors exposed to hypoxia; Cors: Cerebral organoids; CYR61: Cysteine-rich angiogenic inducer 61; DAPI: 4,6-diamidino-2-phenylindole; DEG: Differentially expressed gene; EGM2: Endothelial growth medium-2; FVC: FVCors control; FVH: FVCors exposed to hypoxia; FVCors: Fused vascularized cerebral organoids; GFP: Green fluorescent protein; HDGF: Hepatoma-derived growth factor; HIF-1 α : Hypoxia-inducible factor 1 α ; vOrganoids: Vascularized organoids.

neural crest-derived intermediate cells (melanocyte-like cells), and epithelial cells within a unified platform. Finally, all fabrication processes must satisfy biocompatibility requirements, ensuring minimal cellular stress during scaffold construction and vascular network assembly.

These five interconnected and stringent criteria collectively indicate that simply transplanting vascularization protocols optimized for other organoid systems (e.g., brain organoids) is unlikely to succeed. Instead, it necessitates the development of a comprehensive engineering solution tailored to the unique demands of the inner ear, integrating advanced biomaterial design, high-precision 3D bioprinting, and temporally controlled growth factor delivery strategies.

3.2. Vascularization strategies and inner ear organoid construction

3.2.1. Hypoxia

Research has shown that hypoxia plays a critical role in regulating vascular growth.⁴⁵ Hypoxic conditions enhance the expression of hypoxia-inducible factor-1 α (HIF-1 α), inducing secretion of factors such as vascular endothelial growth factor (VEGF) to mediate processes including sprouting angiogenesis. When integrated with microfluidic systems, oxygen concentrations can be switched at minute-scale intervals, enabling dynamic modulation of signaling pathways.⁴⁶ Studies have demonstrated that hypoxia supports endothelial cell differentiation, with established standardized protocols and extensive research foundations.⁴⁷ However, hypoxia-induced vasculature exhibits stochastic network patterning and fails to recapitulate the radially organized architecture characteristic of the stria vascularis. It also demonstrates limited capacity to promote differentiation of non-endothelial cell lineages.

In a recent study, researchers successfully generated fused vascularized cerebral organoids (FVCors) through staged induction of ectodermal and mesodermal lineages. Following hypoxia treatment, the FVCors exhibited significant upregulation of inflammation-related genes. Venn diagram analysis revealed distinct transcriptomic signatures between vascularized and non-vascularized organoids under hypoxic stress. [Figure 3A](#) demonstrates that 343 differentially expressed genes (DEGs) were uniquely upregulated in FVCors following hypoxia. Gene Ontology enrichment analysis identified inflammation-related pathways (e.g., *RELB*, *IRF9*), indicating that hypoxia triggers inflammatory responses in vascularized constructs, which may compromise organoid integrity.

Correspondingly, [Figure 3B](#) shows 404 uniquely downregulated DEGs predominantly associated with cell cycle regulation, suggesting that hypoxia disrupts proliferative capacity in ectoderm-derived tissues. These

findings collectively argue against prolonged hypoxic exposure during inner ear organoid vascularization, as inflammatory activation and cell cycle arrest would impair sensory epithelium development.⁴¹ Concurrently, hypoxic exposure readily induces pro-apoptotic gene expression and triggers excessive reactive oxygen species production, potentially causing irreversible cellular damage.⁴⁸

In summary, while hypoxia may rapidly initiate vasculogenesis during early endothelial proliferation, it presents significant challenges for engineering structurally complex stria vascularis-like tissues.

3.2.2. Growth factor delivery

Studies have shown that angiogenic growth factors play a crucial role in promoting angiogenesis.⁴⁹ Key angiogenic factors (e.g., VEGF, FGF2, IGF) can bind to specific binding sites within the ECM and, under protease-mediated cleavage, be released into the ECM.⁵⁰

In this section, we delineate the roles of critical angiogenic factors in inner ear developmental morphogenesis and discuss the developmental rationale for applying these insights to organoid vascularization approaches. Critically, in the context of inner ear organoid engineering, the selection and delivery of angiogenic factors must account for their pleiotropic roles in otic morphogenesis. FGF signaling exemplifies this dual functionality: during early development, FGF3 and FGF10 signal through FGF receptor (FGFR)-2b to induce otic placode formation from the pre-placodal ectoderm, while at later stages, FGF9 and FGF20 regulate cochlear progenitor proliferation through mesenchymal FGFR1 and FGFR2.^{51,52}

Notably, FGF20 expressed within the Sox2-positive prosensory domain signals to FGFR1 at the lateral edge of the prosensory domain to regulate outer hair cell and supporting cell differentiation,⁵³ whereas FGF8 expressed by inner hair cells signals to FGFR3 in outer hair cells and supporting cells to regulate pillar cell differentiation.⁵⁴ This intricate spatiotemporal regulation underscores that FGF-based vascularization strategies for inner ear organoids must be carefully calibrated to avoid disrupting sensory epithelium patterning.

Vascular endothelial growth factor similarly exhibits inner ear-specific functions beyond canonical angiogenesis. VEGFA is expressed in cochlear structures, including the stria vascularis, spiral ligament, and SGNs. Recent studies have demonstrated that VEGFA165 stimulates cochlear pericytes, rather than endothelial cells, to function as leading tip cells guiding new vascular branch growth in both adult and neonatal mouse cochleae, challenging the prevailing view that endothelial tip cells exclusively guide angiogenesis.⁵⁵ Furthermore, VEGF signaling is required for supporting cell proliferation and hair cell regeneration in

the avian cochlea, indicating that VEGF pathway activation in inner ear organoids may simultaneously influence both vascular network formation and sensory epithelium regeneration.⁵⁶ These findings suggest that VEGF delivery strategies for inner ear organoid vascularization must consider the unique pericyte-dependent angiogenic mechanism of the stria vascularis and the potential effects on hair cell fate.

To overcome the limitations of growth factor-based approaches, transcription factor-driven vascularization has emerged as a promising alternative. The E26 transformation-specific (ETS) transcription factor ETS variant transcription factor 2 (ETV2; also known as ER71) plays a central role in specifying endothelial cell fate during embryonic development and has been successfully employed to vascularize organoids derived from multiple lineages, including cerebral, kidney, and liver organoids. ETV2 overexpression in hPSCs achieves greater than 90% differentiation efficiency toward functional endothelial cells, circumventing the need for complex growth factor cocktails.⁵⁷

Importantly, ETV2-based co-differentiation strategies have been demonstrated to enable orthogonal differentiation of endothelial and parenchymal lineages within a unified culture system in cerebral organoid models. This approach holds potential relevance for inner ear organoids, where coordinated development of the vascular stria and sensory epithelia is essential; however, direct application to inner ear systems remains to be validated.⁵⁸ Recent advances have demonstrated that combining ETV2-induced endothelial cells with tissue-specific progenitors generates vascularized organoids exhibiting improved barrier function, tight junction integrity, and organ-specific endothelial identity.⁵⁹ For inner ear applications, ETV2-based approaches could potentially be integrated with otic induction protocols to achieve synchronous development of BLB components and sensory structures.

Evidence suggests that growth factors at excessively low concentrations inhibit angiogenesis, while high concentrations may lead to unstable vessels or even tumorigenesis.⁴⁸ Therefore, precise regulation of growth factor concentrations is crucial for the application of angiogenic growth factor delivery strategies in promoting vascularization. Notably, to achieve spatiotemporal precision in delivery, growth factor-based strategies are frequently integrated with complementary technologies to promote organoid vascularization.

A study demonstrated that appropriate growth factors can facilitate the construction of vascular networks in ectoderm-derived organoids.⁴² In one approach, cerebral organoids were directly co-cultured with a self-organized vascular bed composed of human umbilical vein endothelial

cells within a microfluidic device. Transcriptomic analysis of the outermost region of the vascularized organoids revealed that the vascular network was primarily restricted to the peripheral zone. The VEGF/HIF-1 α signaling pathway was identified as a central regulator of angiogenesis, involving 108 pathway components. Further screening and functional validation identified CYR61 and HDGF as potent pro-angiogenic factors.⁴²

As shown in [Figure 3C](#), organoids cultured with CYR61 or HDGF exhibited substantially enhanced angiogenic sprouting by day 10 compared to endothelial cell growth medium-2-only controls, with sprouts extending deeper into the organoid interior. This enhanced vascular penetration addresses a key limitation of conventional organoid vascularization—namely, the peripheral confinement of vessel networks—and suggests that targeted growth factor supplementation could facilitate the deep vascular invasion required for stria vascularis reconstruction. This study not only identifies novel candidate factors for enhancing organoid vascularization but also underscores the pivotal role of growth factors in angiogenic processes.⁴²

3.2.3. Vascular cell co-culture strategies

The co-culture strategy involving pro-angiogenic cells—such as endothelial progenitor cells, fibroblasts, supporting cells, and pericytes—is widely employed to promote vascularization in ectoderm-derived organoids. In earlier studies, researchers co-seeded human induced pluripotent stem cells or hESCs together with human umbilical vein endothelial cells onto low-cell-adhesion plates to form embryoid body-like aggregates, which were then transferred into neural induction medium, resulting in vascularized cerebral organoids.

Across over 30 independent experiments, the success rate of organoid vascularization exceeded 95% using four distinct cell lines, demonstrating high reproducibility of the culture protocol. The vascularized organoids exhibited larger sizes and thicker neuroepithelium compared to non-vascularized counterparts. Quantitative analysis ([Figure 3D](#)) revealed significantly reduced cleaved caspase-3-positive cells and HIF-1 α expression in vascularized organoids at day 115, indicating that functional vasculature alleviates hypoxic stress and suppresses apoptosis. Consequently, vascularized organoids achieved substantially larger diameters throughout the culture period ([Figure 3E](#)), demonstrating that vascular integration overcomes the diffusion-limited size constraints of conventional organoid systems—a prerequisite for engineering cochlear-scale constructs.⁴³

In contrast to conventional approaches, research has identified ETV2 as a master regulator of endothelial development. ETV2 activation can reprogram fibroblasts

and hPSCs into endothelial cells.⁶⁰ Cakir *et al.*⁶¹ applied this discovery to human cortical brain organoids by incorporating engineered ETV2-inducible hESCs into aggregates termed embryoid bodies, thereby promoting vascular network formation within cortical organoids. These networks not only expressed molecular markers (e.g., *CLDN5*, *ABCB1*, *GLUT1*) but also facilitated pericyte and astrocyte recruitment, effectively modeling blood–brain barrier architecture. This approach enables the synchronous differentiation of endothelial cells, cortical neurons, and astrocytes, forming claudin-5–expressing blood–brain barrier-like units with high scalability and controlled cellular damage risk. However, the stochastically branched vascular networks fail to establish organotypic radial patterning and lack dynamic regulation of signaling pathways. Integration with 3D-printable vascular-guiding scaffolds may offer promise for achieving spatially precise cellular organization.⁶²

3.2.4. Scaffold-based tissue engineering methods

In recent years, advances in 3D printing technology have garnered increasing attention for the engineering of vascularized scaffolds. As noted in existing reviews, endothelial cells are highly sensitive to the biophysical properties of scaffolds. Mechanical stimuli can activate or inhibit relevant signaling pathways, while biomolecular signals may in turn modulate the physical characteristics of the microenvironment, collectively exerting critical influences on vascular development and formation.⁶³ Consequently, significant efforts have been devoted to identifying materials that balance biocompatibility with tunable mechanical properties to optimize vascular scaffold construction. Among these, gelatin methacryloyl (GelMA) has emerged as a promising biomaterial, as demonstrated by previous studies, enabling mechanical controllability via parameter modulation (e.g., stiffness adjustment) and offering novel strategies for engineering vascularized scaffolds.

In 2025, Salehi *et al.*⁴⁴ developed a simple and efficient micromolding technique to structure gelatin hydrogels crosslinked by microbial transglutaminase (mTG), while monitoring the mechanical evolution of the hydrogels and endothelial cell behavior over a 14-day period. The results showed a progressive increase in Young's modulus—from 148.2 kPa to 529.5 kPa over 14 days of incubation (Figure 3F)—demonstrating that mTG-crosslinked gelatin hydrogels undergo controlled stiffening during culture, a property that could be exploited to provide stage-specific mechanical cues during inner ear organoid maturation.

Importantly, surface microgrooves enhanced endothelial cell behavior. Figure 3G shows elevated vinculin expression on structured scaffolds compared with non-structured controls, indicating improved focal adhesion formation,

while Figure 3H shows increased vascular endothelial cadherin expression, confirming enhanced endothelial cell–cell junction assembly. These findings indicate that topographical engineering of hydrogel scaffolds can direct endothelial organization, which is a critical consideration for reconstructing the organized capillary networks of the stria vascularis. The study by Salehi *et al.*⁴⁴ demonstrates that straightforward surface engineering approaches can precisely control the topographical features of gelatin-based hydrogels, thereby effectively guiding endothelial cell behavior and accelerating the formation of functional endothelium.

Notably, as highlighted in existing reviews, a growing number of vascularized scaffolds rely on the high spatial precision of 3D printing technology to achieve controlled branching of vascular networks. In analogous neural organoid systems, Cai *et al.*⁶² employed 3D printing to fabricate vascular network-inspired diffusible scaffolds for midbrain organoids. These scaffolds feature a parallel-arranged, hollow, meshed tubular network that achieves precisely controlled diffusion distances, thereby enabling efficient mass transport and effectively addressing the issue of necrosis prevalent in conventional organoids. By precisely tuning the mechanical architecture and biological properties of advanced vascular scaffolds, it is possible to integrate them effectively with vascularization strategies to meet the requirements of vascularized tissue scaffolds.⁶⁴ Topics related to 3D printing will be further discussed later in this review.

Collectively, the inherent limitations of monolithic vascularization strategies necessitate combinatorial engineering of microvascular networks. According to advanced research, next-generation solutions will likely integrate tunable scaffolds with dynamically adjustable viscoelasticity via photo-responsive crosslinkers^{65,66}, biomaterial libraries optimized for angiogenic biocompatibility⁸, and microfluidic organ-chips enabling precise spatiotemporal control over VEGF/Notch signaling cascades.⁶⁷ Such integrated platforms hold significant potential for recapitulating the stria vascularis's ion-transport functionality while ensuring metabolic sustainability through enhanced perfusion.

To construct an inner ear-specific vessel network, scaffolds must replicate the anisotropic stiffness, longitudinal gradient characteristics, and frequency-specific compliance of the tectorial and basilar membranes observed *in vivo* to faithfully replicate cochlear mechanoelectrical transmission.^{68,69} These mechanical characteristics are essential for simulating the traveling wave dynamics of sound-induced basilar membrane vibrations. Therefore, scaffolds used to construct vascularized inner ear organoids must possess excellent mechanical properties

and biocompatibility, enabling the formation of three-layered cellular architectures and allowing appropriate vascular spacing. Optimized biomaterials, high-precision 3D bioprinting, and multicellular co-culture systems hold promise for providing new solutions for vascularization in inner ear construction.

3.2.5. Integration of vascularization strategies: Toward a unified framework for inner ear organoids

While individual vascularization strategies each possess distinct advantages, their isolated implementation often fails to meet the complex requirements of inner ear organoid engineering. The stria vascularis requires not only functional vasculature but also a precise three-layered cellular architecture and ion transport capacity. Consequently, the integration of complementary technologies represents a critical path forward.

There are three integration modalities that merit consideration. First, microfluidic–bioprinting convergence addresses a fundamental mismatch: bioprinting creates geometrically defined microchannels but lacks hemodynamic cues, whereas microfluidics provides dynamic flow but cannot generate complex intraluminal architectures. Their integration yields perfusable scaffolds that enable continuous nutrient delivery and shear stress application, essential for endothelial maturation.^{70,71}

Second, the bioprinting–co-culture combination reconciles spatial precision with cellular complexity. Multi-nozzle systems deposit distinct cell populations at predetermined coordinates, achieving layer-by-layer stria cell organization unattainable through either approach alone.⁵⁸

Third, microfluidic–co-culture integration orchestrates vascular development temporally: controlled shear stress and VEGF/Notch gradients guide the transition from active angiogenesis to stable, mature vasculature—a process impossible under static conditions. Importantly, these pairwise combinations can be hierarchically assembled into comprehensive tripartite platforms that simultaneously address spatial organization, cellular diversity, and dynamic microenvironmental control.⁷²

Emerging evidence suggests that assembloid strategies, wherein independently matured vascular and sensory organoids are fused, may confer superior vascular stability compared to conventional co-differentiation approaches.⁷³ Pre-formed vascular networks within vascular spheroids exhibit greater structural integrity, enhanced pericyte coverage, and reduced regression rates relative to de novo-assembled vessels in co-culture systems. Applying these integrated approaches to stria vascularis engineering

requires careful consideration of its unique architecture. The tripartite cellular arrangement, comprising marginal cells facing the endolymph, intermediate cells interfacing with capillaries, and basal cells forming tight junction barriers, necessitates layered bioprinting with cell type-specific positioning. Microfluidic systems can subsequently establish the ionic gradients essential for endocochlear potential generation while providing shear stress for endothelial maturation.

Future protocols should systematically evaluate how these integrated platforms influence both vascular functionality and sensory epithelium development, with particular attention to whether combined approaches better preserve the self-organization capacity that is essential for authentic inner ear tissue formation.

In summary, this section systematically evaluated vascularization strategies through the lens of inner ear-specific requirements, departing from generalized organoid reviews by proposing a “vascularization–mechanotransduction–ion homeostasis” framework. We emphasized that, unlike cerebral organoids, where vasculature primarily serves metabolic functions, inner ear vascularization must recapitulate the ion-transporting epithelium of the stria vascularis. By comparing quantitative parameters across hypoxia induction, growth factor delivery, cell co-culture, and scaffold-based approaches (Table 1), we found that no single strategy adequately addresses the tripartite cellular architecture and tight junction requirements of the BLB. Consequently, we advocate for integrated platforms combining bioprinting precision, microfluidic dynamic control, and assembloid-based pre-vascularization to achieve both structural fidelity and functional ion transport capacity.

3.3. Bioprinting-enabled vascularization for stria vascularis engineering

The preceding sections have examined vascularization strategies and their individual merits; however, a critical gap remains between conceptual frameworks and practical implementation. Specifically, achieving the tripartite cellular architecture of the stria vascularis, with its precise marginal–intermediate–basal cell arrangement spanning merely 30–40 μm in total thickness, demands fabrication precision beyond the capabilities of conventional self-organization approaches. 3D bioprinting, with its capacity for spatially controlled cell deposition and scaffold architecture, offers a pathway to bridge this gap. This section synthesizes recent advances in bioprinting technologies and evaluates their potential application to stria vascularis reconstruction, focusing on three interconnected challenges: multi-cellular positioning, perfusable microchannel fabrication, and gradient-driven barrier maturation.

3.3.1. Multi-nozzle printing for the positioning of marginal–intermediate–basal cells

The stria vascularis exhibits a precisely stratified architecture wherein marginal cells, intermediate cells, and basal cells are arranged in defined spatial relationships that are essential for ion transport function.³⁸ Recapitulating this organization demands printing technologies capable of depositing distinct cell populations with micrometer-scale precision. Multi-nozzle extrusion bioprinting has emerged as a promising approach for achieving such spatial control. Kang *et al.*⁷⁴ demonstrated that the Integrated Tissue and Organ Printer system, equipped with multiple dispensing modules, could fabricate heterogeneous tissue constructs with 50 μm resolution while maintaining >95% cell viability. Brassard *et al.*⁷¹ further demonstrated that bioprinting could direct organoid self-organization by controlling initial cell positioning, achieving reproducible tissue patterning across multiple organ types.

Building on these precedents, we propose a three-nozzle configuration designed to recapitulate the tripartite cellular architecture of the stria vascularis. Nozzle 1 would deposit marginal cell-laden GelMA bioink formulated to support epithelial phenotype maintenance and expression of ion transport machinery, including Na^+/K^+ -ATPase and potassium voltage-gated channel (KCN) subfamily Q member 1/KCN subfamily E regulatory unit 1 channels, which are essential for K^+ secretion into the endolymph. The selection of GelMA for this application reflects its demonstrated compatibility with epithelial cell culture and

tunable mechanical properties.⁷⁵

Nozzle 2 would dispense intermediate cells of neural crest origin, which express the inwardly rectifying potassium channel Kir4.1, using a softer bioink formulation. This reduced stiffness is intended to accommodate the distinct mechanosensitivity of neural crest-derived lineages, which typically require compliant substrates for optimal survival and function. Nozzle 3 would print basal cells in a bioink optimized to promote tight junction assembly, particularly claudin-11 expression, critical for establishing the paracellular barrier that prevents ionic equilibration between vascular and endolymphatic compartments. The rationale for employing GelMA-based bioinks across this configuration is supported by Xia *et al.*³⁴, who demonstrated that GelMA/hyaluronic acid (HA)/arginine–glycine–aspartic acid peptide motif (RGD) hydrogels support cochlear progenitor cell expansion and differentiation through stiffness-dependent mechanotransduction. Furthermore, the capacity to modulate GelMA stiffness across a physiologically relevant range (0.5–50 kPa) by varying polymer concentration and crosslinking density enables the creation of cell type-specific mechanical microenvironments within a unified printing system.

3.3.2. Sacrificial ink strategies for perfusable microchannel networks

The stria vascularis microvasculature comprises capillaries with diameters ranging from 5 to 20 μm and length densities of 360–484 mm/mm³.⁴⁰ Engineering fine-scale perfusable

Table 1. Quantitative parameters of different vascularization strategies

| Strategy | Vascular density | Diffusion limitation | Vessel diameter | Perfusion capability | References |
|------------------------|---|--|---|---|------------|
| Hypoxia | Variable; enhances vascular endothelial growth factor expression but produces stochastic network patterning | 100–200 μm (unchanged without perfusion) | 10–50 μm (capillary-like) | Non-perfusable without integration | 45 |
| Growth factor delivery | Controllable via concentration gradients | 100–200 μm without functional vessels | Variable; gradient-dependent | Non-perfusable intrinsically | 49,50 |
| Cell co-culture | Moderate; $\sim 4.2\times$ tissue density in pancreatic islets, with $\sim 50\%$ coverage achievable | Improved to 250–500 μm with network formation | Capillary-like sprouts (10–30 μm) | Limited; requires additional flow systems | 60,61 |
| Scaffold-based | Moderate–high; controllable channel spacing | 200–400 μm between channels | 30–1,500 μm (technique-dependent) | Variable; 50–100% perfusable channels | 44,62,64 |

networks requires sacrificial bioprinting strategies, wherein fugitive inks are deposited to define channel geometry and subsequently removed to yield hollow conduits. Miller *et al.*⁷⁶ pioneered carbohydrate glass-based sacrificial printing, demonstrating that rapidly dissolving sugar filaments could template vascular networks capable of supporting endothelial cell seeding and perfusion. Kolesky *et al.*⁷⁷ advanced this approach using Pluronic F127, a thermoreversible hydrogel that liquefies below 4 °C, enabling fabrication of interconnected microchannels (150–600 µm diameter) within GelMA matrices that support more than seven-day perfusion culture. More recently, Lee *et al.*⁷⁸ developed the freeform reversible embedding of suspended hydrogels (FRESH) technique using gelatin microparticle support baths, achieving channel resolutions approaching 20 µm, which falls within the range required for stria vascularis capillary mimicry.

Recent advances have improved sacrificial bioprinting resolution. The sacrificial writing into functional tissue method developed by Skylar-Scott *et al.*⁷⁹ enables the fabrication of perfusable vascular channels within densely packed organoid matrices containing approximately 200 million cells/mL, demonstrating the feasibility of creating complex vascular architectures in high-cellular-density constructs. The FRESH technique has achieved 20-µm filament resolution through pH-controlled collagen gelation, enabling the fabrication of complex anatomical structures, including multiscale vasculature.⁷⁸ While these technical advances represent substantial progress, achieving stria vascularis-scale microvasculature (5–20 µm capillary diameters) remains a significant engineering challenge, requiring further resolution improvements.

Collectively, the integration of multi-nozzle printing and sacrificial templating strategies presents a technically demanding but potentially transformative approach to stria vascularis engineering. While individual components have been validated in other organoid systems, their coordinated application to inner ear constructs remains unexplored. Key technical milestones include: achieving sub-20 µm channel resolution that matches native capillary dimensions, establishing stable tri-layered cell configurations over extended culture periods (>30 days), and demonstrating functional ion transport capacity via electrophysiological validation. The convergence of these bioprinting innovations with the vascularization principles outlined in **Sections 2.1** and **2.2** establishes the foundation for the biomaterial considerations addressed in the following section.

4. Inner ear organoids based on three-dimensional bioprinting

The field has transitioned from 2D to 3D culture since Koehler *et al.*²⁹ first generated inner ear organoids.

3D bioprinting technology provides new methods for organoid maturation.⁸⁰ Multi-cell printing can mimic tissue complexity through 3D bioprinting's remarkable control over cell and material organization and demonstrates significant development potential.⁸¹ The most popular 3D bioprinting techniques include extrusion-based, inkjet-based, stereolithography (SLA), and laser-assisted bioprinting, together with the recently emerged volumetric bioprinting (VBP). The comparison of scaffold-based and scaffold-free 3D bioprinting methods for inner ear organoids will be discussed in the following section, along with the potential applications of different bioinks and future developments for 3D printing inner ear systems. [Table 2](#) provides comparison of different 3D bioprinting modalities which will be discussed below.

4.1. Three-dimensional bioprinting modalities

According to the American Society for Testing and Materials standards and established classifications, current 3D bioprinting technologies can be broadly categorized into three groups: extrusion-based, jetting-based, and vat photopolymerization-based approaches, with several emerging modalities further expanding this landscape.^{80,82}

Extrusion-based bioprinting, the most widely adopted modality in both academic research and commercial platforms, operates by dispensing a continuous filament of bioink through a nozzle via pneumatic pressure, piston, or screw-driven mechanisms.^{80,81} This method accommodates bioinks with a broad range of viscosities, including cell-laden hydrogels at physiologically relevant cell densities, and supports multi-nozzle configurations that enable the simultaneous deposition of multiple cell types and biomaterials.^{74,82}

Inkjet-based bioprinting, by contrast, generates discrete picoliter-scale droplets through thermal, piezoelectric, or electromagnetic actuation. While offering higher resolution and printing speed, inkjet systems are limited to low-viscosity bioinks and relatively low cell concentrations, which limits their utility for constructing thick, cell-dense tissues.⁸²

Light-based bioprinting modalities, including SLA, digital light processing (DLP), and two-photon polymerization, employ photon energy to selectively crosslink photosensitive bioinks.^{80,83} SLA achieves high resolution through point-by-point laser scanning but at relatively slow fabrication speeds, whereas DLP cures entire layers simultaneously via projected light patterns, offering a favorable balance between resolution and throughput. Laser-assisted bioprinting, also termed laser-induced forward transfer, deposits bioink droplets from a donor ribbon to a receiving substrate using focused laser pulses, achieving high resolution and cell viability without nozzle-

related clogging.⁸⁰

More recently, VBP has emerged as a paradigm-shifting approach that fundamentally departs from the layer-by-layer fabrication logic shared by all preceding modalities. VBP fabricates the entire 3D object simultaneously by projecting computed tomographic light patterns into a rotating reservoir of photosensitive, cell-laden resin. As the cumulative light dose selectively exceeds the polymerization threshold at target coordinates within the volume, the desired geometry solidifies in a single continuous exposure lasting only seconds to tens of seconds. Bernal *et al.*⁸⁴ subsequently applied this principle to organoid bioprinting, fabricating centimeter-scale, organoid-laden GelMA constructs in under 20 s while preserving superior cell viability and organoid morphology compared to extrusion-based methods.

The most prominent advantage of VBP over other bioprinting technologies lies in the elimination of nozzle-mediated shear stress during fabrication. Xia *et al.*³⁴ demonstrated that cochlear progenitor cell (CPC) fate is regulated by matrix stiffness, and studies have found that VBP circumvents this risk entirely: organoids fabricated via VBP exhibit metabolic activity and live-to-dead ratios comparable to non-printed cast controls, confirming that the printing process itself imposes minimal perturbation to the cellular mechanobiological state.⁸⁴ Furthermore, the ultra-rapid solidification inherent to VBP substantially reduces the duration of environmental perturbation during organoid construction, potentially enhancing protocol reproducibility.

In the context of inner ear organoid engineering, extrusion-based bioprinting has been the predominant technique employed to date. This preference reflects its compatibility with the hydrogel bioinks commonly used in otic research, which is critical for reconstructing the stratified cellular architecture of cochlear structures such as the organ of Corti and the stria vascularis. The multi-nozzle capability of extrusion systems is especially relevant for depositing distinct cell-laden bioinks to recapitulate the marginal-intermediate-basal cell layering discussed in Section 3.3.1. Nevertheless, the limited resolution of extrusion-based methods presents challenges for replicating the fine microstructural features of the inner ear, such as the 5–20 μm capillaries of the stria vascularis, suggesting that higher-resolution modalities, such as DLP or two-photon polymerization, may complement extrusion-based approaches in future studies.

4.2. Inner ear organoid three-dimensional construction from scaffold-free to scaffold-based system

The transition from scaffold-free to scaffold-based inner

ear organoid culture represents a paradigm shift from relying solely on biochemical cues to harnessing the mechanobiological principles that govern otic development. Three fundamental principles have emerged from this transition: (i) matrix stiffness exerts biphasic control over cochlear progenitor cell fate; (ii) scaffold microarchitecture directly influences cellular organization and adhesion; and (iii) chemically defined matrices are essential for reproducibility and clinical translation.

4.2.1. Matrix stiffness as a master regulator of otic progenitor fate

A central insight from scaffold-based engineering is that ECM rigidity critically determines whether CPCs proliferate or differentiate. Earlier studies identified ECM composition, scaffold architecture, and biomaterial properties as key parameters for 3D inner ear organoid construction, yet the mechanistic basis for these effects remained unclear.⁸³ This gap was addressed by Xia *et al.*³⁴, who diverged from conventional Matrigel-based approaches to fabricate tunable-stiffness culture systems using GelMA–HA–RGD composite hydrogels. Within this scaffold, GelMA provided exceptional mechanical tunability, HA enhanced physical stability and degradation resistance, and RGD motifs improved biocompatibility.^{80,81,85}

Their conceptual framework is schematically depicted in Figure 4A, illustrating the biphasic relationship between matrix stiffness and CPC fate: soft hydrogels (1.5 kPa) activate integrin $\alpha 3$ /Yes-associated protein mechanosensing to promote progenitor expansion, while stiffer matrices (5 kPa) trigger Ca^{2+} -dependent extracellular signal-regulated protein kinase/Krüppel-like factor 2 signaling that drives hair cell differentiation. This mechanistic model provides actionable design parameters for bioink formulation in inner ear bioprinting applications.

Quantitative analysis confirmed this biphasic model. Figure 4B shows that 5-ethynyl-2'-deoxyuridine-positive cell percentages peaked in 1.5 kPa hydrogels, indicating maximal proliferative capacity at intermediate stiffness. Correspondingly, ATP-based viability assays (Figure 4C) demonstrated that CPCs in 1.5 kPa GelMA–HA–RGD hydrogels maintained significantly higher viability than those in Matrigel or stiffer formulations, establishing 1.5 kPa as the optimal stiffness for the expansion phase of inner ear organoid culture.

During the differentiation phase, however, stiffer matrices proved superior. Figure 4D demonstrates that *Atoh1*-EGFP expression, a direct readout of hair cell fate commitment, was maximal in 5 kPa hydrogels. This was corroborated by *Myo7a* immunostaining (Figure 4E), which showed the highest percentage of mature hair cell marker expression under stiff conditions.

Together, these findings establish a two-stage culture protocol wherein initial expansion in soft matrices, followed by transfer to stiff substrates, optimizes both yield and differentiation efficiency. This stiffness–fate relationship carries important implications for vascularized inner ear organoid engineering. Since endothelial cells similarly respond to substrate mechanics, with softer matrices favoring sprouting angiogenesis and stiffer substrates promoting vessel stabilization, scaffold design must balance the mechanical requirements of both sensory epithelium and vascular development. The GelMA-based platform's tunability (0.5–50 kPa range) provides sufficient flexibility to address this challenge through spatially and/or temporally graded stiffness profiles.

4.2.2. Scaffold microarchitecture governs cellular organization

Beyond bulk mechanical properties, scaffold microstructure—including pore size, surface topology, and interconnectivity—critically influences cell seeding efficiency, nutrient transport, and tissue organization. This principle is exemplified by the work of Zhang *et al.*⁸⁶, who developed polyvinyl alcohol (PVA)/GelMA/sodium alginate (SA) printed scaffolds for neonatal mouse organ of Corti organoids. The selection of scaffold materials and their concentration ratios not only influences rigidity but also determines pore size and surface roughness, which directly govern cellular behavior within the scaffold.

Scanning electron microscopy revealed that increasing PVA concentration systematically altered scaffold microarchitecture. Figure 4F shows representative images demonstrating progressively reduced pore dimensions with higher PVA content, while quantitative analysis (Figure 4G) confirmed pore sizes ranging from 150–200 μm depending on the formulation. This tunability enables optimization of scaffold porosity for specific applications: larger pores facilitate cell seeding and nutrient diffusion, whereas smaller pores may enhance mechanical stability and cell retention. This structural configuration, characterized by interconnected porosity and elevated surface topography, has been shown to facilitate cellular adhesion, proliferation, and growth on the scaffolds.⁸⁶

Building on this foundation, Wang *et al.*⁸⁷ systematically compared scaffolds printed with bioinks containing varying concentrations of GelMA and SA. Scaffolds composed of 8% GelMA/2% SA exhibited reduced pore dimensions and enhanced surface compactness, whereas 7% GelMA/1% SA formulations exhibited larger, more homogeneous internal porosity with superior fluid absorption capacity. Comparative analysis of cellular morphology (Figure 4H) revealed that inner ear stem cells cultured on 3D bioscaffolds (panels B, B', B'') exhibited more homogeneous cytoskeletal distribution and expanded spreading areas

compared to conventional well plate cultures (panels A, A', A''). The enhanced cell spreading indicates superior cell–matrix adhesion on 3D scaffolds, which is critical for mechanotransduction-mediated differentiation signaling in cochlear progenitors. Stem cell adhesion further confirmed the scaffolds' favorable biocompatibility profile.⁸⁷

4.2.3. Transition to chemically defined matrices for clinical translation

While Matrigel has enabled foundational discoveries in inner ear organoid biology, its undefined composition, batch variability, and xenogenic origin present insurmountable barriers to clinical translation. This limitation has driven the development of chemically defined alternatives that maintain biological functionality while enabling reproducible manufacturing. Arkenberg *et al.*⁶⁵ demonstrated the utility of chemically defined click hydrogels for inner ear applications. Using thiol–norbornene photopolymerization for primary crosslinking, followed by aldehyde–hydrazine click chemistry for secondary network formation, they created dynamically crosslinked hydrogels with precisely tunable mechanical properties. Mechanical characterization (Figure 4I) showed that gelatin–norbornene–carbohydrazide hydrogels crosslinked with 0.1 or 1 wt% 4-arm poly(ethylene glycol) thiol achieved elastic moduli comparable to Matrigel, providing a xeno-free alternative with equivalent mechanical properties. Critically, live/dead staining (Figure 4J) confirmed high cell viability across conditions, while vesicle quantification revealed that soft gelatin–norbornene–carbohydrazide hydrogels supported otic vesicle formation comparable to Matrigel in both number (Figure 4K) and diameter (Figure 4L), establishing these defined matrices as viable substitutes for clinical translation.

4.2.4. Clinical translation considerations for biomaterial selection

Beyond the fundamental requirements of biocompatibility and printability, clinical translation of bioprinted inner ear constructs demands rigorous evaluation of manufacturing and regulatory parameters that are often overlooked in preclinical studies. Batch-to-batch consistency represents a critical quality attribute for regulatory approval. Matrigel exhibits significant variability in both mechanical and biochemical properties within and between production lots, with proteomic analyses identifying more than 14,000 unique peptides and nearly 2,000 unique proteins whose relative abundances vary across batches.⁸⁵

Chemically defined alternatives, such as GelMA-based hydrogels, can achieve improved batch consistency when synthesized under controlled conditions with standardized methacrylation degrees.⁷⁵ For clinical manufacturing, the implementation of Good Manufacturing Practice-

compliant synthesis protocols and comprehensive release testing is essential. Long-term mechanical evolution post-implantation directly influences organoid integration and function. The progressive stiffening observed in mTG-crosslinked gelatin hydrogels (148 kPa to 530 kPa over 14 days, as shown in Figure 3F)⁴⁴ may initially support organoid development but could eventually compromise the mechanical compliance required for hair cell

mechanotransduction. Designing scaffolds with controlled degradation profiles that maintain optimal stiffness ranges throughout the therapeutic window requires careful alignment of hydrolytic or enzymatic degradation rates with tissue maturation kinetics.

Finally, degradation product toxicity within the cochlear microenvironment warrants careful consideration. The

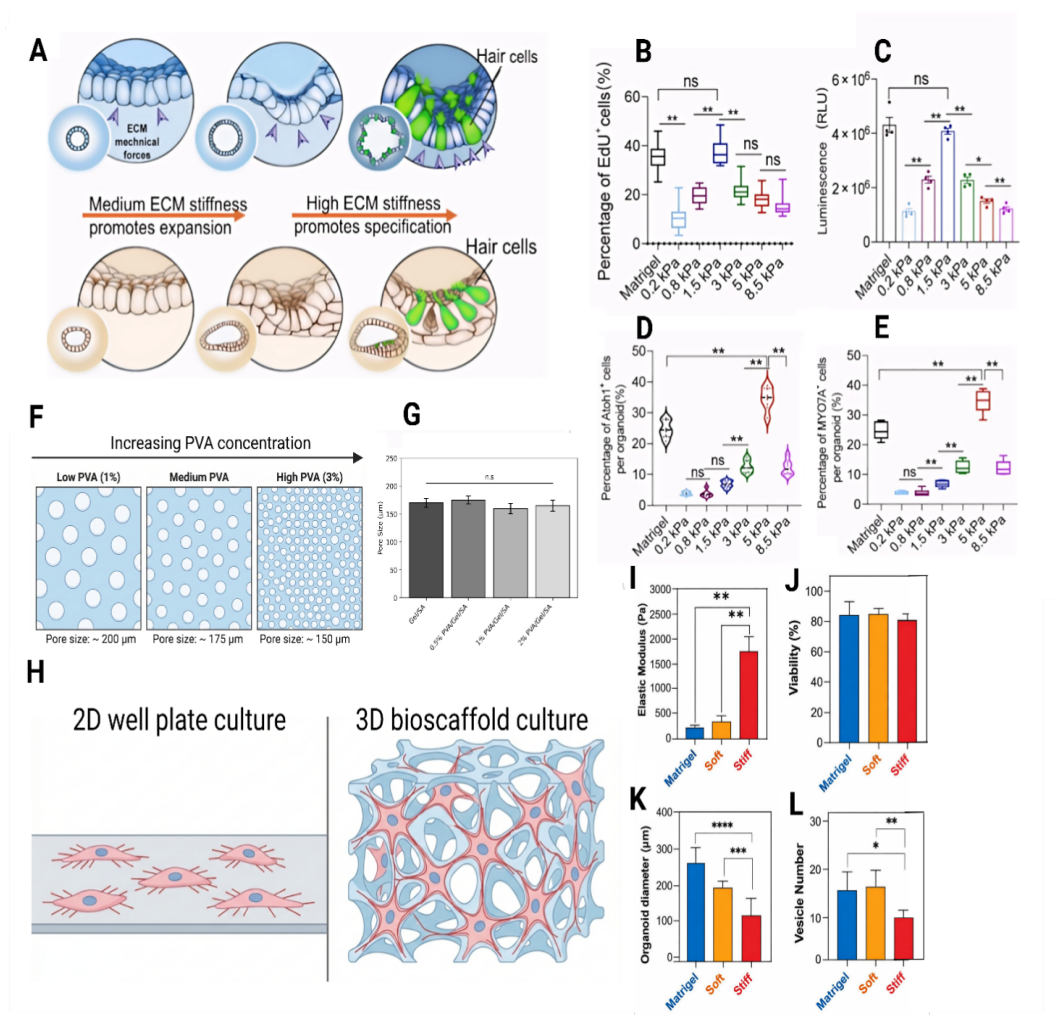


Figure 4. 3D construction of scaffold and inner ear organoids. (A) Schematic of gradient hydrogel stiffness modulating staged sensory epithelium formation in organoids. Reproduced with permission from Xia *et al.*³⁴ Copyright © 2023, American Association for the Advancement of Science. (B) Percentage of EdU-positive cells per organoid. Reproduced with permission from Xia *et al.*³⁴ Copyright © 2023, American Association for the Advancement of Science. (C) Luminescent cell viability assay of CPCs cultured in Matrigel and hydrogels with varying stiffness. Reproduced with permission from Xia *et al.*³⁴ Copyright © 2023, American Association for the Advancement of Science. (D) Percentage of *Atoh1*-EGFP-positive cells per organoid. Reproduced with permission from Xia *et al.*³⁴ Copyright © 2023, American Association for the Advancement of Science. (E) Percentage of *Myo7a*-positive cells per organoid. Reproduced with permission from Xia *et al.*³⁴ Copyright © 2023, American Association for the Advancement of Science. (F) PVA/Gel/SA scaffolds with varying PVA concentrations. Created using BioRender. Li, F. (2026) <https://BioRender.com/qfw2sdp>. (G) Pore size of PVA/Gel/SA scaffolds. (H) Adhesion of inner ear stem cells cultured on scaffold surfaces in well plates. Created using BioRender. Li, F. (2026) <https://BioRender.com/pw61u9j>. (I) Elastic moduli of Matrigel and GelNB-CH hydrogels under non-dynamic conditions. (J) Semi-quantitative analysis of live/dead staining results. (K) Vesicle number under tested conditions. (L) Vesicle diameter under tested conditions. Notes: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ indicate statistical significance; whereas “ns” indicates not significance. Abbreviations: 3D: Three-dimensional; CH: Carbohydrazide; CPC: Cochlear progenitor cell; EdU: 5-Ethynyl-2'-deoxyuridine; Gel: Gelatin; NB: Norbornene; PVA: Polyvinyl alcohol; SA: Sodium alginate.

Table 2. Comparison of three-dimensional bioprinting modalities and their characteristics relevant to inner ear organoid engineering

| Bioprinting modality | Working principle | Key advantages | Limitations | References |
|-----------------------------|---|--|---|------------|
| Extrusion-based bioprinting | Continuous filament deposition through a nozzle via pneumatic, piston, or screw-driven forces | Compatible with high-viscosity bioinks (including GelMA, alginate, and collagen); supports multi-nozzle configurations for heterogeneous tissue construction; high cell-loading capacity | Limited resolution compared to light-based methods; shear stress during extrusion may compromise cell viability; slower printing speed for large constructs | 80,81 |
| Inkjet-based bioprinting | Discrete droplet deposition via thermal, piezoelectric, or electromagnetic actuators | High printing speed; good resolution; low cost and broad availability; capable of generating concentration gradients | Restricted to low-viscosity bioinks (<10 mPa-s); limited cell density; nozzle clogging at higher cell concentrations; poor structural integrity for thick constructs | 82 |
| Stereolithography | Point-by-point photopolymerization of photosensitive resin via UV or visible laser scanning | High spatial resolution; smooth surface finish; ability to fabricate complex microarchitectures | Slow fabrication due to raster scanning; limited bioink selection (requires photocrosslinkable materials); potential UV-induced cytotoxicity; low cell density | 80,83 |
| Laser-assisted bioprinting | Laser-induced forward transfer of bioink droplets from a donor ribbon to a substrate | Nozzle-free (no clogging); high resolution; compatible with a wide range of bioink viscosities; high cell viability | Low throughput; high equipment cost; difficulty in scaling up; limited to thin 2D/quasi-3D constructs | 80 |
| Volumetric bioprinting | Simultaneous whole-volume photopolymerization via computed tomographic light projections | Ultra-fast printing (seconds to minutes); minimal shear stress; enables printing within pre-existing structures (e.g., organoids) | Emerging technology with limited standardization; requires optically transparent, photocrosslinkable bioinks; resolution constrained by light scattering at high cell | 84 |

Abbreviations: 2D: Two-dimensional; 3D: Three-dimensional; GelMA: Gelatin methacryloyl; UV: Ultraviolet.

inner ear's limited capacity for waste clearance, due to the BLB and minimal lymphatic drainage, may result in local accumulation of scaffold degradation products.³⁷ Systematic cytotoxicity screening of degradation products against cochlear cell types, combined with pharmacokinetic modeling of clearance rates, should inform biomaterial selection for clinical applications.

4.2.5. Critical evaluation and future directions

Despite these advancements, current scaffold-based approaches have several limitations. First, the stiffness–fate relationships established by Xia *et al.*³⁴ derive primarily from mouse CPCs; whether human otic progenitors exhibit identical mechanosensitivity thresholds remains unverified. Second, existing studies have focused predominantly on sensory epithelium development, with limited attention to how scaffold properties influence the concurrent differentiation of strial and neural lineages required for functional organoids. Third, the integration of vascular networks within mechanically tuned scaffolds introduces additional complexity: perfusion-induced shear stress may alter the effective mechanical environment experienced by embedded cells, potentially modifying optimal stiffness

parameters.

In summary, scaffold-based 3D printing technology (Table 3) offers significant advantages over scaffold-free approaches, including enhanced structural precision, superior control over ECM composition, provision of mechanical support, and facilitation of substance exchange. The convergence of tunable biomaterials, high-resolution bioprinting, and mechanobiological understanding establishes a foundation for engineering inner ear constructs with unprecedented structural and functional fidelity.

4.3. Inner ear organoid three-dimensional construction with comparison of different biomaterials

Three-dimensional bioprinting enables precise spatial control over biological materials, biochemicals, and living cells to construct tissue-like structures.⁸⁰ In this process, bioinks—composed of living cells and bioactive components—are deposited layer-by-layer to form predefined architectures mimicking native tissues.⁸² However, a critical challenge lies in replicating the dynamic ECM microenvironment, which plays an important role in

Table 3. Recent advancements in inner ear organoid three-dimensional construction: Comparative analysis of methods, advantages, and limitations

| Biomaterials | Technical parameters | Construction methods | Key findings | Advantages | Limitations | References |
|----------------------------------|--|---|---|--|--|------------|
| MXene–Matrigel | Concentration of MXene: 300 µg/mL | Isolated mouse cochlear cells were suspended in Matrigel or MXene–Matrigel and allowed to solidify at 37°C to establish a 3D culture environment | The MXene–Matrigel composite significantly enhanced functional hair cell regeneration and neurite reinnervation by potentiating the mTOR signaling pathway | Enhanced electrical conductivity; promotes neural connectivity; simple preparation | Retains Matrigel's batch variability; limited mechanical tunability | 86 |
| GelMA–HA–RGD 3D hydrogel network | Elastic modulus: 0.5–2.0 kPa; HA concentration: 1 mg/mL; RGD peptide density: 0.5 mM | Elevating GelMA concentration directly enhances hydrogel stiffness by augmenting crosslinking density, as quantified through storage modulus (G') measurements | Matrix rigidity tuning revealed biphasic CPC regulation: moderate stiffness enhanced proliferation through ITGA3/YAP mechanosensing, while higher stiffness favored hair cell differentiation via Ca^{2+} -dependent ERK/KLF2 activation | Chemically defined; tunable stiffness; mechanistic insights into mechanotransduction | Complex multi-component preparation; requires optimization of component ratios | 34 |
| Graphene oxide medium | Thickness of single graphene layer: 1.3–1.4 nm; size: 1.23 µm | Graphene oxide was blended into differentiation medium at varying concentrations, with microwell chips employed as distinct culture matrices to investigate modulatory effects on inner ear organoid maturation under controlled conditions | Graphene oxide enhances the differentiation efficiency of hair cells and supporting cells. Concurrently, microwell chips optimize graphene-organoid interfacial interactions, thereby consistently improving organoid viability and functional maturation | Enhanced differentiation efficiency; improved organoid uniformity; good biocompatibility | Potential cytotoxicity at high concentrations; limited scalability | 66 |

(Cont'd...)

Table 3. (Continued)

| Biomaterials | Technical parameters | Construction methods | Key findings | Advantages | Limitations | References |
|---|--|--|--|---|---|------------|
| PVA/GelMA/SA composite hydrogel | PVA: 1%; scaffold porosity: 150–200 μm ; neomycin: 200 mg/kg; BBR: 50mg/kg | Employing extrusion-based bioprinting with low-temperature deposition modeling. Corti organ tissue was directly seeded onto prefabricated PVA/Gel/SA scaffolds for organoid culture | BBR significantly upregulated p-AKT expression, thereby promoting the expression of anti-apoptotic proteins such as Bcl-2. This cascade effectively blocked caspase-3-mediated apoptotic signaling, ultimately inhibiting neomycin-induced hair cell apoptosis | Good mechanical robustness; tunable porosity; suitable for drug screening | Requires optimization of concentration ratios; moderate resolution | 88 |
| Decellularized cochlear ECM-functionalized hydrogel | Eliminating EGF and IGF-1 from the conventional cytokine protocol, excluding VPA, adding DMH1 and prostratin to regulate the signaling pathway | A unified culture medium enables autonomous cellular self-organization through spatiotemporal signaling control, achieving single-step differentiation from progenitors to functional hair cells | The organoids exhibit a solid 3D architecture with cellular organization recapitulating the native hair cell-supporting cell alternating pattern, accompanied by enhanced cellular diversity. This platform has been validated to model ototoxic damage and screen otoprotective compounds | Simplified one-step protocol; high reproducibility; clinically relevant | Limited structural complexity; requires further validation for clinical translation | 8 |
| GelMA/stearyl acrylate/PVA composite hydrogel | Scaffold: 20 mm \times 20 mm \times 2 mm network; porosity: 150–200 μm ; elasticity modulus: 0.5–2kPa | Scaffolds were fabricated via extrusion-based bioprinting, followed by photocrosslinking, onto which isolated Corti organs were seeded while preserving native cell–cell interactions | Solid organoids recapitulate cochlear cellular spatial organization, demonstrating potential as a standardized ototoxicity model and scalable platform for regenerative therapies | Preserves native tissue architecture; scalable; standardized platform | Limited vascularization; requires optimization for long-term culture | 87 |

Abbreviations: 3D: Three-dimensional; BBR: berberine; CPC: Cochlear progenitor cell; ECM: Extracellular matrix; EGF: Epidermal growth factor; GelMA: Gelatin methacryloyl; HA: Hyaluronic acid; IGF-1: Insulin-like growth factor 1; ITGA3: Integrin alpha 3; mTOR: Mammalian target of rapamycin; p-AKT: Phosphorylated protein kinase B; PVA: Polyvinyl alcohol; RGD: Arginine–glycine–aspartic acid peptide motif; SA: Sodium alginate; YAP: Yes-associated protein.

governing stem cell proliferation, differentiation, and tissue maturation. ECM contains a variety of proteins organized into intricate topographic patterns that direct cells toward their appropriate phenotype.⁸⁹ Additionally, ECM is crucial for cell adhesion, proliferation, and migration, and it helps modulate inhibitory signaling.⁹⁰

The ECM not only provides structural support but also delivers biophysical (e.g., stiffness, topology) and biochemical cues (e.g., protein composition), which are crucial in determining the fate of stem cells. Furthermore, evidence confirms that conductive materials modulate organoid development, neurogenesis, and synaptic reinnervation through bioelectrical signal transmission in neural tissues.^{11,91} Consequently, bioinks, which are used to construct and mimic ECM *in vitro*, must possess well-balanced biocompatibility, tunable mechanical properties, controlled biodegradability, and electrical conductivity to effectively promote hair cell differentiation and synaptogenesis with SGNs.

This section discusses recent advances in biomaterials (Figure 5), including Matrigel, gelatin, GelMA, SA, HA, RGD, polypyrrole–polydopamine (PPY–PDA), and PVA, focusing on these critical parameters to inform rational biomaterial selection for inner ear organoid engineering.

Matrigel is a type of basement membrane ECM that is derived from the secretion of Engelbreth–Holm–Swarm mouse sarcoma cells.⁹² Numerous studies have shown that Matrigel encourages the differentiation of multiple cell types by activating or inhibiting the BMP, TGF- β , FGF, and Wnt signaling pathways via recombinant proteins and small chemical inhibitors, which have been applied in the field of inner ear organoid research.⁹³ However, Matrigel possesses an intricate and variable composition, which raises the risk of immunogenicity. Additionally, Matrigel presents immunogenic hazards in clinical settings involving humans because it is derived from mouse cells.⁹⁴ Although its mechanical characteristics, such as stiffness and pore size, have a significant impact on cell and tissue development, they are also uneven throughout the gel and difficult to separate from biochemical influences. Therefore, the matrix limits the investigation of ECM mechanical signals' impact on organoid morphogenesis produced by CPCs due to its low controllability over physical and biochemical properties. While Matrigel retains unique merits in supporting organoid self-organization, its intrinsic limitations in tunability and batch-to-batch consistency have driven a paradigm shift toward chemically defined alternatives. This critical transition to xeno-free matrices enables precise control over viscoelasticity and biochemical gradients, a prerequisite for reproducible, precision organoid engineering.

As a denatured collagen derivative, gelatin has emerged

as a promising bioink candidate for inner ear organoid engineering due to its exceptional biocompatibility, intrinsic RGD motifs for cell adhesion, and cost-effective processability.⁹⁵ Critically, gelatin demonstrates low immunogenic reactivity in mammalian systems, with *in vivo* studies revealing minimal antibody responses in both animal models and human tissues.⁹⁶ This biological inertness, combined with its semi-structured macromolecular architecture containing cell-instructive domains, positions gelatin as a favorable substrate for mechanosensitive cochlear cell development. Nevertheless, inherent limitations in thermostability and shape fidelity necessitate chemical modifications for advanced bioprinting applications. To address this issue, Arkenberg *et al.*⁶⁵ employed thiol–norbornene photopolymerization to achieve primary crosslinking of hydrogels encapsulating differentiated organoids, followed by the implementation of an aldehyde–hydrazine click reaction to induce secondary crosslinking within the aggregate-laden networks. Experimental results demonstrate that this chemically defined, dynamically crosslinked click hydrogel exhibits a dense network architecture, tunable mechanical stiffness, significantly enhances cell viability and otic vesicle formation, and represents a viable alternative to Matrigel for inner ear tissue engineering applications.⁶⁵

In addition, methacrylation of lysine residues has become a cornerstone strategy for creating dimensionally stable constructs. Under photoinitiator activation, this process generates GelMA—an ultraviolet-crosslinkable hydrogel that preserves native ECM biomimicry while introducing tunable mechanical properties. As a subclass of hydrogels characterized by >90% water content and interpenetrating polymer networks, GelMA-based matrices uniquely combine low immunoreactivity with bioactive signaling capabilities, particularly through matrix metalloproteinase (MMP)–responsive peptide motifs that facilitate dynamic cell–matrix interactions.⁷⁵

Recent breakthroughs highlight GelMA's transformative potential in auditory regeneration. Li *et al.*⁹⁷ demonstrated that cochlear organoids cultured in GelMA hydrogels exhibited upregulated MMP9-mediated ECM remodeling, with pharmacological MMP inhibition significantly impairing organoid morphogenesis, suggesting MMP activity as a critical regulator of inner ear development. Building on this mechanistic insight, Wang *et al.*⁸⁷ engineered a hybrid GelMA/SA bioink system that achieved high structural fidelity in 3D-printed Corti organoids, establishing a novel platform for hearing loss therapeutics screening. As natural polymers, gelatin and SA possess high biocompatibility, low cytotoxicity, and strong adhesive properties. By utilizing 3D printing to fabricate PVA/gelatin/SA composite scaffolds, researchers successfully engineered Corti organoids, providing an effective platform

for investigating therapeutic interventions against hearing loss.⁸⁸ PVA, known for its biocompatibility, biodegradability, and mechanical robustness with flexibility, was shown to significantly enhance the strength of gelatin/SA composite scaffolds.

Early studies indicated that HA-mediated pressure drives semicircular canal formation, while RGD motifs, by mimicking adhesive proteins within the ECM, enhance cellular attachment and spreading efficiency.⁹⁸ Researchers incorporated HA and RGD into a GelMA hydrogel system, demonstrating that HA increases water absorption and reduces pore size, thereby improving hydrogel mechanical stability. The integrated GelMA–HA–RGD hydrogel system achieved tunable mechanical stiffness and enhanced expansion and differentiation efficiency of sensory epithelium.³⁴

Additionally, researchers successfully engineered a hydrogel system with excellent biocompatibility and electrical conductivity by incorporating PPY–PDA nanowires into Matrigel hydrogels. The PPY–PDA nanowires were synthesized through oxidative polymerization of dopamine and pyrrole, initiated using ammonium persulfate. Researchers encapsulated PPY–PDA–Matrigel composites within microfluidic chips and seeded mouse cochlear sensory epithelial cells to enhance cochlear organoid differentiation under low-frequency electroacoustic stimulation (EAS). Results demonstrated that PPY–PDA–Matrigel synergized with EAS to

significantly promote inner ear progenitor cell proliferation and hair cell regeneration. The derived organoids exhibited high sensitivity to ototoxic drugs, indicating that electroacoustic-responsive cochlear chips enable high-throughput screening of otoprotective compounds. This platform provides a robust and promising *in vitro* system for evaluating inner ear therapeutics.⁶⁷

Notably, precise control of biomaterial concentration emerges as a critical parameter across applications. Empirical evidence indicates that supraoptimal concentrations elicit significant cytotoxicity, suppressing stem cell proliferation and differentiation.⁸⁶ Conversely, when biomaterial concentration governs hydrogel stiffness, it mechanistically modulates Notch/Wnt pathway activation via alterations in pore architecture (e.g., size, density), elastic modulus, and hydration capacity, ultimately dictating cellular behavior.^{34,87}

In summary, biomaterial selection is critical for inner ear organoid bioprinting, as it establishes mechanistic links between scaffold properties and otic morphogenesis. We progressed from describing scaffold-free versus scaffold-based approaches to evaluating specific bioink formulations, highlighting how tunable stiffness modulates Notch/Wnt signaling and consequent hair cell differentiation. Critically, we identified the transition from Matrigel-dependent protocols toward chemically defined alternatives (e.g., GelMA, gelatin-norbornene hydrogels) as essential for clinical translation, given Matrigel's batch variability and xenogenic risks. The comparative analysis of biomaterial

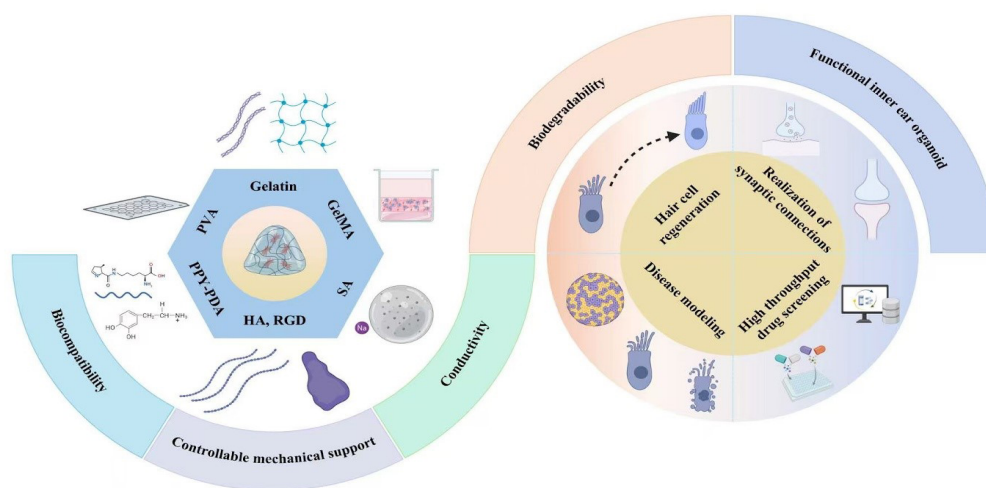


Figure 5. Overview of different biomaterials for constructing inner ear organoids. Created using BioRender. Estella, A. (2026) <https://BioRender.com/bbh61r5>.

Abbreviations: GelMA: Gelatin methacryloyl; HA: Hyaluronic acid; PPY–PDA: Polypyrrole–polydopamine; PVA: Polyvinyl alcohol; RGD: Arginine–glycine–aspartic acid peptide motif; SA: Sodium alginate.

parameters, including MMP-responsive degradation, RGD-mediated adhesion, and electroconductive modifications, provides a framework for selecting appropriate matrices based on experimental objectives, whether mechanistic studies requiring precise stiffness control or drug screening demanding reproducible high-throughput platforms. The comparative properties of commonly used biomaterials for inner ear organoid engineering, including their advantages, limitations, and optimal applications, are summarized in Table 4.

4.4. Computational approaches for optimizing bioprinted inner ear organoid engineering

The increasing complexity of bioprinted tissue constructs necessitates computational tools capable of predicting and optimizing both fabrication parameters and post-printing biological performance. Computational fluid dynamics (CFD) and artificial intelligence (AI) approaches have emerged as powerful complements to experimental optimization, offering predictive capabilities that accelerate the design-build-test cycle while reducing material waste and experimental iterations.

4.4.1. Computational fluid dynamics-assisted prediction of mechanical parameters

As an emerging computational technique, CFD can be employed to optimize mechanical parameters, which is particularly critical for shear stress-sensitive CPCs.³⁴ Fareez *et al.*⁹⁹ indicated that CFD enables optimization of printability, cell viability, and structural fidelity without extensive experimental iteration by computational analysis of flow velocity, shear stress, and pressure distribution within bioprinter nozzles. For instance, Goto-Silva *et al.*¹⁰⁰ employed CFD simulations to quantitatively predict physical parameters, including flow velocity and shear stress, in brain organoid culture systems while minimizing experimental burden, thereby demonstrating the value of CFD as a predictive tool. Similar CFD analyses could inform the selection of dynamic culture systems for inner ear organoids, particularly given the documented mechanosensitivity of cochlear progenitor cells.

4.4.2. Integration of artificial intelligence toward emerging opportunities

Traditionally, researchers have required months or even years of iterative experimentation to identify suitable culture conditions. The integration of AI, particularly Bayesian optimization and reinforcement learning, has transformed this process from blind trial-and-error into a directed, intelligent search. For example, Kanda *et al.*¹⁰¹ developed an AI system integrating experimental robotics and Bayesian optimization to autonomously identify optimal differentiation conditions from 200 million possible parameter combinations. Through

three consecutive optimization rounds, the AI system successfully identified culture conditions that reproducibly differentiate stem cells into retinal pigment epithelial cells with quality suitable for cell therapy research. Furthermore, AI applications reduce operator-dependent variability, providing a unified evaluation framework. Abdul *et al.*¹⁰² established a deep learning model, D-CryptO, to achieve automatic classification of organoid opacity in brightfield images, replacing substantial manual interpretation. Their study demonstrated the capability of AI algorithms to automatically assess the internal structural complexity of organoids.

5. Application of inner ear organoids

Inner ear organoids, as *in vitro* systems that recapitulate inner ear development and physiological environments, have broad applications in high-throughput drug screening, gene therapy, regenerative medicine, and disease modeling. Continued research is essential to advance organoid maturation and clinical translation, ultimately improving therapeutic outcomes for patients with hearing loss.

5.1. High-throughput drug screening

High-throughput drug screening enables the rapid identification of compounds with ototoxic, protective, or regenerative properties. Traditional animal models used for drug testing, such as mice and zebrafish, are easy to handle and readily available but present limitations, including ethical concerns and genomic differences.¹⁰³ Additionally, zebrafish lack a stria vascularis and cochlea, and their hair cells possess regenerative capabilities, in contrast to the limited regenerative capacity of mammalian inner ear hair cells.

In contrast, cochlear organoids offer superior biomimicry with fewer ethical constraints. Furthermore, individual sensitivity to the same drug may vary,¹⁰⁴ and organoids derived from patient-specific stem cells can assess drug responsiveness, enabling a more personalized treatment approach. In 2021, Liu *et al.*¹⁰⁵ established a high-throughput cochlear organoid platform and identified regorafenib, a VEGF receptor inhibitor, as an effective compound for promoting hair cell differentiation and facilitating the reprogramming and maturation of neomycin-damaged hair cells.

In 2024, Chai *et al.*⁶⁷ incorporated PPY-PDA into Matrigel and integrated cochlear organoids with a microfluidic chip to construct a cochlear-on-a-chip platform. The platform architecture is schematically illustrated in Figure 6A. PPY-PDA conductive nanowires embedded within Matrigel provide electrical conductivity, while microfluidic channels enable controlled drug delivery. Integration with cochlear implant-derived EAS creates a physiologically relevant screening environment

Table 4. Comparative analysis of biomaterial properties and applications for inner ear organoid engineering

| Biomaterial | Advantages | Disadvantages | Batch consistency | Sterilization method | Degradation timeline | Degradation product concerns | References |
|------------------|--|--|----------------------------|---|---|---|------------|
| Matrigel | High biocompatibility; supports self-organization; contains >1,800 native ECM proteins; activates BMP, TGF- β , EGF, Wnt pathways | Batch variability; undefined composition; xenogenic origin (immunogenic risk); low mechanical tunability; uneven stiffness/pore distribution | Poor | Not autoclavable; cold filtration only | Variable (weeks to months) | Xenogenic protein fragments; immunogenic risk | 92–94 |
| Gelatin | Excellent biocompatibility; intrinsic RGD motifs for cell adhesion; low immunogenicity; cost-effective; semi-structured ECM-like domains | Poor thermostability; limited shape fidelity; requires chemical modification for bioprinting | Moderate | Gamma irradiation affects properties | 2–4 weeks (enzyme dependent) | Biocompatible peptides | 95,96 |
| GelMA | High mechanical tunability (0.5–50 kPa); UV-crosslinkable; MMP-responsive for dynamic cell–matrix interactions; preserves ECM biomimicry; >90% water content | UV crosslinking may affect cell viability; requires a photoinitiator; limited conductivity | Good | Aseptic photocrosslinking | Tunable | Methacrylic acid; requires dose assessment | 75,97 |
| GelMA–HA–RGD | Tunable stiffness; enhanced water absorption; improved mechanical stability; RGD motifs enhance cell adhesion; defined composition | Complex preparation; requires optimization of component ratios | Moderate (multi-component) | Aseptic photocrosslinking | Tunable | HA fragments (generally well-tolerated) | 34 |
| PPY–PDA/Matrigel | High electrical conductivity; supports bioelectrical signaling; promotes synaptic reinnervation; compatible with EAS | Limited mechanical control; retains Matrigel's batch variability | Poor (Matrigel-dependent) | UV/ethanol affects conductivity; sterile filtration of components; aseptic assembly | PPY/PDA: non-biodegradable to slow; Matrigel: weeks to months | PPY persists in tissue; PDA degrades to dopamine (biocompatible neurotransmitter); Matrigel xenogenic fragments | 67 |
| PVA/GelMA/SA | Good mechanical robustness; biodegradable; tunable porosity (150–200 μ m); enhanced scaffold stability | Requires optimization of concentration ratios; moderate tunability | Moderate | Moderate | SA: ionic dissolution; GelMA: enzymatic | Alginate oligomers; calcium chelation effects | 88 |

(Cont'd...)

Table 4. (Continued)

| Biomaterial | Disadvantages | Disadvantages | Batch consistency | Sterilization method | Degradation timeline | Degradation product concerns | References |
|----------------------------|---|--|--|--|--|---|------------|
| Click hydrogels (GelNB-CH) | Chemically defined; xeno-free; dynamically crosslinkable; tunable mechanical properties; high reproducibility | Requires specialized chemistry (thiol-norbornene); newer technology with less validation | Good to excellent (synthetic, defined) | Sterile filtration (0.22 µm); UV/visible light photocrosslinking | Tunable (days to weeks); ester hydrolysis-dependent ($k = 0.011 - 0.027 \text{ day}^{-1}$); MMP-sensitive if peptide crosslinkers used | Thioether bonds; biocompatible peptide fragments; PEG (well-tolerated); gelatin fragments | 65 |

Abbreviations: BMP: Bone morphogenetic protein; EAS: Electroacoustic stimulation; FGF: Fibroblast growth factor; GelMA: Gelatin methacryloyl; GelNB-CH: Gelatin-norbornene-carbohydrazide hydrogel; HA: Hyaluronic acid; MMP: Matrix metalloproteinase; PEG: Polyethylene glycol; PPY-PDA: Polypyrrole-polydopamine; RGD: Arginine-glycine-aspartic acid peptide motif; TGF-β: Transforming growth factor-β; UV: Ultraviolet.

that recapitulates the electromechanical milieu of the native cochlea, representing a critical advance over static culture systems for evaluating otoprotective compounds. Using this platform, the researchers demonstrated the protective effect of the antioxidant α-lipoic acid on cisplatin-treated cochlear organoids, establishing a robust *in vitro* system for inner ear drug evaluation.⁶⁷

In 2025, Qin *et al.*⁸ established a one-step, self-organizing inner ear organoid system and demonstrated its sensitivity to ototoxic antibiotics and chemotherapeutic agents. Specifically, they validated the protective effect of dabrafenib against gentamicin-induced hair cell damage, highlighting the platform's broad applicability for drug screening.

5.2. Disease modeling

Traditional deafness research primarily relies on animal models, each with distinct advantages and limitations.¹⁰⁶ Inner ear organoid models, which recapitulate the cellular composition and genetic lineage of the human inner ear, provide a powerful platform for investigating deafness-associated signaling pathways and pathogenic mechanisms. Currently, two methods are used to model genetic deafness *in vitro*: (i) introducing deafness-associated mutations into wild-type ESCs via gene editing, and (ii) reprogramming somatic cells from patients with hereditary deafness into iPSCs, followed by differentiation into inner ear organoids.^{107,108}

In 2018, Zhong *et al.*¹⁰⁹ introduced *Barhl1*-targeted knockout embryonic stem cell lines into inner ear organoids and found that *Barhl1* deficiency led to the downregulation of hair cell-specific genes and upregulation of non-sensory cell markers. As illustrated in Figure 6B, *Barhl1*-knockout organoids exhibited decreased expression of hair cell-specific transcripts (*Myo7a*, *Pou4f3*) with concurrent upregulation of supporting cell markers, demonstrating that *Barhl1* functions as a transcriptional switch between sensory and non-sensory fates. This organoid-based disease model thus provides mechanistic insight and a platform for screening compounds that may compensate for *Barhl1* deficiency. These findings clarify the molecular mechanism underlying *Barhl1*-mediated hair cell differentiation and suggest potential therapeutic strategies for deafness associated with *Barhl1* mutations.

In 2019, Tang *et al.*¹¹⁰ developed an inner ear organoid model with *Tmprss3* mutations and observed increased expression of the apoptotic marker *Caspase3*. Additionally, they reported reduced BK channel expression and disrupted calcium homeostasis in *Tmprss3*-knockout hair cells. The proposed pathogenic mechanism is summarized in Figure 6C. *Tmprss3* mutations impair BK channel function, disrupt calcium homeostasis, and trigger caspase-3-mediated

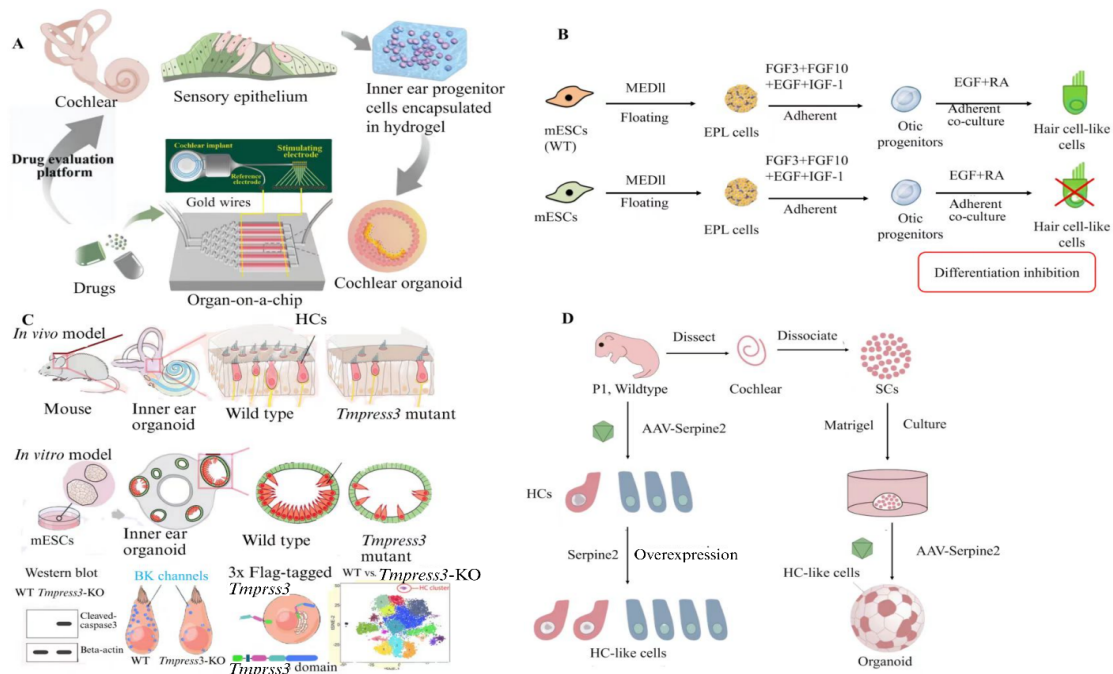


Figure 6. Applications of inner ear organoids. (A) Schematic of cochlear organoids integrated with a conductive hydrogel biohybrid system and cochlear implant-derived electroacoustic stimulation for cochlea-on-a-chip construction and high-throughput drug screening. (B) *In vitro* modeling of hearing impairment caused by *Barhl1* mutation. (C) *In vitro* modeling of hearing loss associated with *Tmprss3* mutation. (D) Overexpression of *Serpine2* promotes hair cell regeneration. Created using BioRender. Li, F. (2026) <https://BioRender.com/z8a83au>.

Abbreviations: AAV, adeno-associated virus; EGF: Epidermal growth factor; EPL: Early primitive ectoderm-like cell; FGF: Fibroblastic growth factor; HC: Hair cell; KO, knockout; MED: Medium; mESC: Mouse embryonic stem cell; RA: Retinoic acid; SC: Stem cell; WT: Wide type.

apoptosis. This organoid model elucidated the molecular basis of *Tmprss3*-associated deafness and identified calcium signaling as a potential therapeutic target. Notably, they also highlighted limitations of inner ear organoids, including developmental instability associated with 2D culture conditions and variability in induction efficiency across cell lines. Future integration of 3D printing, microfluidic systems, and organ-on-a-chip technologies may further enhance organoid stability and functional maturation.

5.3. Hair cell regeneration

It is generally accepted that mammalian hair cells cannot regenerate after injury. However, studies have shown that mammalian supporting cells have the potential to differentiate into hair cells, providing new avenues for hair cell regeneration therapies for hearing loss.^{111,112} By utilizing inner ear organoids, researchers have identified additional signaling pathways related to inner ear development, and these mechanisms offer potential for hair cell regeneration.

In 2024, Sun *et al.*¹¹³ cultured neonatal mouse cochlear epithelial cells in Matrigel to construct inner ear organoids and used adeno-associated virus inner ear vectors to deliver the *Serpine2* gene to supporting cells. They

observed upregulation of cell cycle-related gene expression and significant increases in both the number and size of organoids, indicating that *Serpine2* overexpression promotes the proliferation of inner ear supporting cells. The researchers also found that *Serpine2* overexpression enhanced the mRNA expression of downstream target genes in the canonical Wnt/ β -catenin signaling pathway, suggesting that *Serpine2* may promote hair cell regeneration by activating the Wnt signaling pathway. The proposed regenerative mechanism is depicted in Figure 6D: adeno-associated virus inner ear-mediated *Serpine2* overexpression in supporting cells activates canonical Wnt/ β -catenin signaling, promoting cell cycle re-entry and subsequent transdifferentiation toward hair cell fates. This finding suggests *Serpine2* as a potential gene therapy target for hearing restoration.

In 2020, Li *et al.*¹¹⁴ constructed cochlear organoids by culturing cochlear supporting cells from neonatal mice. They found that a reduction in LIN28B—mammalian target of rapamycin complex 1 (mTORC1) activity accelerated the developmental decline of supporting cell plasticity, whereas overexpression of LIN28B prevented this decline and enabled maturing supporting cells to activate a progenitor-

like state, thereby responding to regenerative cues and generating new hair cells. Ultimately, the researchers used rapamycin to inhibit mTORC1 and discovered that LIN28B-induced reprogramming of supporting cells was dependent on the mTORC1 signaling pathway. An increasing body of research has demonstrated the broad applications and potential of organoids in regenerative medicine, and future precise regulation of spatiotemporal signals during inner ear development may become key to improving organoid culture.

Critically, the long-term survival and functional integration of regenerated hair cells will require adequate metabolic support that current avascular organoid systems cannot provide. The vascularization strategies discussed in **Section 3** thus represent not merely an enhancement but a prerequisite for achieving clinically meaningful regeneration. Future protocols should therefore integrate regenerative stimulation with vascularization engineering, potentially through sequential or concurrent delivery of pro-regenerative and pro-angiogenic factors within bioprinted scaffolds. Such integrated approaches may ultimately enable the generation of vascularized, innervated cochlear constructs containing functional regenerated hair cells.

6. Challenges and perspectives

Inner ear organoids have emerged as promising platforms for disease modeling, drug screening, and regenerative medicine. Current protocols achieve robust differentiation of hair cells and neuronal lineages with conserved developmental kinetics that recapitulate human inner ear ontogenesis. Nevertheless, critical challenges remain, limiting clinical translation. This section systematically analyzes these limitations, identifies their mechanistic root causes, and proposes concrete strategies with testable hypotheses for future investigation.

6.1. Overcoming the diffusion barrier by bioprinting-enabled vascularization for organoid scaling

Current inner ear organoids rarely exceed 500 μm in diameter, with cellular necrosis consistently observed in constructs larger than 200–300 μm due to insufficient oxygen and nutrient penetration.^{36,114} This size constraint represents a fundamental biophysical limitation rather than a biological one: oxygen consumption by metabolically active cells exceeds passive diffusion supply beyond approximately 150–200 μm , creating hypoxic cores that trigger apoptotic cascades.³⁵ Unlike native tissues possessing perfused vascular networks, organoids rely exclusively on surface diffusion, fundamentally limiting their scalable growth. The consequences extend beyond viability concerns, including constraints on recapitulating the 35 mm spiral length and the tonotopically graded hair

cell distribution essential for frequency-specific hearing function.

The root cause of this limitation lies in the absence of convective nutrient transport mechanisms within organoid cores. While the stria vascularis *in vivo* maintains metabolically demanding ion transport through an intricate capillary network with vessel densities of 360–484 mm/mm^3 ,⁴⁰ current organoid systems lack any vascular equivalent. This deficiency creates not only nutritional bottlenecks but also prevents the establishment of the BLB, whose tight junction proteins are essential for maintaining the ionic gradients underlying the endocochlear potential.

Several strategies have been explored in other organoid systems to address this limitation. Vascular network-inspired diffusible scaffolds, featuring parallel hollow tubular networks spaced at 200–400 μm intervals, have maintained >90% cell viability throughout 2-mm-thick midbrain organoid masses.⁶² The FRESH bioprinting technique has achieved channel resolutions approaching 20 μm in cardiac tissue constructs, which falls within the range of native stria capillary diameters (5–20 μm). Sacrificial templating with thermoreversible Pluronic F127 inks has enabled fabrication of interconnected microchannels supporting extended perfusion culture in kidney organoids.⁷⁷ Whether these approaches can be successfully translated to inner ear organoids remains to be experimentally determined.

Key considerations include the unique metabolic demands of mechanosensory hair cells, the requirement for BLB reconstruction rather than simple nutrient delivery, and potential interference of scaffold materials with mechanotransduction. Future studies systematically optimizing vascular network-inspired diffusible scaffold parameters for cochlear constructs represent an important direction for advancing inner ear organoid technology. Moreover, emerging technologies in dynamically responsive hydrogels offer new possibilities for addressing the temporal requirements of inner ear organoid vascularization. Two-photon crosslinking of photosensitive hydrogels enables dynamic fabrication of instructive hydrogel elements within pre-existing organ-like cultures at any time during culture.¹¹⁵

Additionally, programmable niche platforms exploiting wavelength-specific photochemical reactions have demonstrated independent control over pendant ligand photoconjugation and crosslink photocleavage, enabling dynamic modulation of 3D cellular microenvironments.¹¹⁶ Translation of these approaches to inner ear constructs could enable the biphasic stiffness protocol (soft for expansion, stiff for differentiation) identified by Xia *et al.*³⁴ to be implemented within a single continuous culture system rather than requiring physical transfer between matrices.

6.2. Reconstructing the endocochlear potential: From structural to functional maturation

Even if scaling challenges are overcome, a more fundamental limitation confronts inner ear organoid technology: no current system demonstrates measurable endocochlear potential. This +80–100 mV ionic gradient between endolymph and perilymph compartments drives the mechanotransduction currents essential for hair cell function,³⁸ yet remains entirely absent in organoid cultures. Without this potential, organoid hair cells, despite expressing appropriate markers and exhibiting stereociliary bundles, cannot achieve the functional maturity required for meaningful drug screening or disease modeling applications.

The mechanistic root cause lies in the absence of the tripartite cellular architecture of the stria vascularis. Generation of the endocochlear potential requires the coordinated activity of marginal cells expressing Na⁺/K⁺-ATPase and KCNQ1/KCNE1 channels, intermediate cells derived from neural crest containing Kir4.1 potassium channels, and basal cells forming tight junctions via claudin-11.³⁷ Current self-organization protocols successfully generate sensory epithelium but fail to specify these stria cell lineages, which derive from distinct developmental origins—neural crest for intermediate cells and otic mesenchyme for basal cells—thereby requiring separate induction pathways that are not incorporated into existing differentiation schemes.

Several approaches have been explored in analogous systems to address multi-lineage co-differentiation challenges. ETV2-driven strategies have achieved >90% endothelial differentiation efficiency in cerebral organoids while enabling orthogonal differentiation of parenchymal lineages.⁵⁴ Gradient bioprinting has established VEGF-to-platelet-derived growth factor gradients within GelMA hydrogels, directing endothelial cell migration and enhancing barrier function, with transepithelial/transendothelial electrical resistance values increasing three-fold compared to non-patterned controls in kidney organoids.⁵⁵ Neural crest-derived intermediate cell precursors have been generated through Wnt activation and endothelin-3 supplementation in melanocyte differentiation protocols,³⁷ though their application to inner ear constructs has not been reported.

Translation of these strategies to inner ear organoids faces unique challenges. The precise spatial arrangement of marginal–intermediate–basal cells, spanning merely 30–40 μm total thickness, demands fabrication precision at the limit of current bioprinting capabilities.⁷⁴ Furthermore, functional validation requires establishing measurable transepithelial potentials, a technical challenge that has not been systematically addressed in the organoid literature. Future advances in multi-lineage co-differentiation

protocols, combined with high-resolution bioprinting, may enable reconstruction of stria vascularis architecture, potentially opening avenues for modeling stria-dependent pathologies such as Pendred syndrome and age-related metabolic presbycusis.

6.3. Enabling clinical translation through standardization and organ-on-chip integration

The preceding challenges of scaling and functional maturation must ultimately be addressed within a framework that enables clinical translation. Currently, significant variability exists across laboratories in organoid induction efficiency and functional endpoints, stemming from undefined Matrigel composition, subjective morphological criteria, and the absence of quantitative quality control metrics. Standardization requires two parallel advances directly relevant to bioprinting–vascularization integration. First, the transition from Matrigel to chemically defined matrices, such as the GelMA–HA–RGD hydrogel system, eliminates protocol variability while providing tunable mechanical properties (0.5–50 kPa) essential for bioprinting applications.³⁴ The quantitative stiffness–fate relationships established for otic progenitors provide reproducible design parameters for bioink formulation.³² Second, organ-on-chip integration enables real-time monitoring of vascularized constructs. Embedding microsensors for continuous transepithelial/transendothelial electrical resistance measurement provides immediate feedback on BLB integrity during maturation. The cochlea-on-chip platform demonstrated by Hu *et al.*⁶⁷, featuring conductive PPY–PDA substrates with integrated electrical stimulation, exemplifies how microfluidic perfusion systems can simultaneously support vascularization while enabling functional validation through electroacoustic stimulation.

Recent multi-center reproducibility studies in other organoid systems have demonstrated that chemically defined matrices combined with standardized differentiation protocols can reduce inter-laboratory variability from >50% to <20% for key functional readouts.⁹⁸ Whether similar standardization can be achieved for inner ear organoids remains an important question for the field. With the aid of AI integration, establishing consensus quality control metrics and reference standards represents a prerequisite for future regulatory approval and clinical application of bioprinted inner ear constructs.

7. Conclusion

This article reviews the development of inner ear organoid construction, focusing on the systematic integration of inner ear physiological and anatomical characteristics. It elaborates on the application of 3D printing and vascularization strategies in constructing inner ear

organoids. Finally, the review discusses the clinical applications, challenges, and future prospects of inner ear organoids.

Acknowledgments

None.

Funding

The work was financially supported by the National Natural Science Foundation of China (22174121, 22211530067, and T2250710180), the 2022 Natural Science Foundation of Guangdong Provincial Basic and Applied Basic Research Fund (Guangdong Hybridio, the Guangdong Pearl River Talent Program (2021CX02Y066), Shenzhen Bay Open Laboratory Fund 2021, the CUHKSZ-Boyalife Joint Laboratory Fund, the University Development Fund (UDF01002012), 1+1+1 CUHK-CUHK(SZ)-GDSTC Joint Collaboration Fund, and the Guangdong Basic Research Center of Excellence for Aggregate Science Fund.

Conflict of interest

The authors declare they have no competing interests.

Author contributions

Conceptualization: Guozhen Liu

Funding acquisition: Guozhen Liu

Methodology: Jianxia Chen

Project administration: Guozhen Liu

Supervision: Guozhen Liu

Visualization: Fuzheng Li, Kexin Yang

Writing—original draft: Fuzheng Li

Writing—review & editing: Fuzheng Li, Kexin Yang, Mohsen Asadnia, Jianxia Chen

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Data will be available upon request.

References

1. Qi J, Zhang L, Wang X, *et al.* Modeling, applications and challenges of inner ear organoid. *Smart Med.* 2024;3(1):e20230028.
doi: 10.1002/SMMD.20230028
2. Wagner EL, Shin J-B. Mechanisms of hair cell damage and repair. *Trends Neurosci.* 2019;42(6):414-424.
doi: 10.1016/j.tins.2019.03.006
3. World Health Organization. Deafness and hearing loss. Available from: <https://www.who.int/news-room/fact-sheets/detail/deafness-and-hearing-loss> [Last accessed on August 6, 2025]
4. Lu Y-C, Wu C-C, Yang T-H, *et al.* Differences in the pathogenicity of the p. H723R mutation of the common deafness-associated SLC26A4 gene in humans and mice. *PLoS ONE.* 2013;8(6):e64906.
doi: 10.1371/journal.pone.0064906
5. Yi Z, Wang X, Yin G, Sun Y. The Blood-Labyrinth Barrier: Non-Invasive Delivery Strategies for Inner Ear Drug Delivery. *Pharmaceutics.* 2025;17(4):482.
doi: 10.3390/pharmaceutics17040482
6. Koehler KR, Nie J, Longworth-Mills E, *et al.* Generation of inner ear organoids containing functional hair cells from human pluripotent stem cells. *Nat Biotechnol.* 2017;35(6):583-589.
doi: 10.1038/nbt.3840
7. Castaño-González K, Köppl C, Pyott SJ. The crucial role of diverse animal models to investigate cochlear aging and hearing loss. *Hear Res.* 2024;445:108989.
doi: 10.1016/j.heares.2024.108989
8. Qin X, Fu L, Li C, Tan X, Yin X. Optimized inner ear organoids for efficient hair cell generation and ototoxicity response modeling. *Sci China Life Sci.* 2025;68(5):1369-1383.
doi: 10.1007/s11427-024-2803-1
9. van der Valk WH, Nist-Lund C, Zhang J, *et al.* Generation and characterization of vestibular inner ear organoids from human pluripotent stem cells. *Nat Protoc.* 2025;21(2):391-428.
doi: 10.1038/s41596-025-01191-3
10. Lou Y, Liu Y, Wu M, Jia G, Xia M, Li W. Inner ear organoids: Recent progress and potential applications. *Fundam Res.* 2025;5(6):2926-2936.
doi: 10.1016/j.fmre.2023.07.013
11. Wang Y, Chen M, Pan Y, Li X, Lou X. Inner Ear Organoid as a Preclinical Model of Hearing Regeneration: Progress and Applications. *Stem Cell Rev Rep.* 2025;21:2031-2042.
doi: 10.1007/s12015-025-10941-5
12. Moeinzaziri F, Zarkesh I, Pooyan P, Nunez DA, Baharvand H. Inner ear organoids: Progress and outlook, with a focus on the vascularization. *FEBS J.* 2022;289(23):7368-7384.
doi: 10.1111/febs.16146
13. Pianigiani G, Roccio M. Inner Ear Organoids: Strengths and Limitations. *J Assoc Res Otolaryngol.* 2024;25(1):5-11.
doi: 10.1007/s10162-024-00929-2
14. Streit A. Origin of the vertebrate inner ear: Evolution and induction of the otic placode. *J Anat.* 2001;199(Pt 1-2):99-103.

- doi: 10.1046/j.1469-7580.2001.19910099.x
15. Choo D. The role of the hindbrain in patterning of the otocyst. *Dev Biol.* 2007;308(2):257-265.
doi: 10.1016/j.ydbio.2007.05.035
 16. Ohyama T, Groves AK, Martin K. The first steps towards hearing: Mechanisms of otic placode induction. *Int J Dev Biol.* 2007;51(6-7):463-472.
doi: 10.1387/ijdb.072320to
 17. Kelly MC, Chen P. Development of form and function in the mammalian cochlea. *Curr Opin Neurobiol.* 2009;19(4):395-401.
doi: 10.1016/j.conb.2009.07.010
 18. Whitfield TT. Development of the inner ear. *Curr Opin Genet Dev.* 2015;32:112-118.
doi: 10.1016/j.gde.2015.02.006
 19. Lim R, Brichta AM. Anatomical and physiological development of the human inner ear. *Hear Res.* 2016;338:9-21.
doi: 10.1016/j.heares.2016.02.004
 20. Köppl C, Wilms V, Russell IJ, Nothwang HG. Evolution of Endolymph Secretion and Endolymphatic Potential Generation in the Vertebrate Inner Ear. *Brain Behav Evol.* 2018;92(1-2):1-31.
doi: 10.1159/000494050
 21. Driver EC, Kelley MW. Development of the cochlea. *Development.* 2020;147(12):dev162263.
doi: 10.1242/dev.162263
 22. Li H, Liu H, Heller S. Pluripotent stem cells from the adult mouse inner ear. *Nat Med.* 2003;9(10):1293-1299.
doi: 10.1038/nm925
 23. Oshima K, Grimm CM, Corrales CE, *et al.* Differential distribution of stem cells in the auditory and vestibular organs of the inner ear. *J Assoc Res Otolaryngol.* 2007;8(1):18-31.
doi: 10.1007/s10162-006-0058-3
 24. Oshima K, Shin K, Diensthuber M, Peng AW, Ricci AJ, Heller S. Mechanosensitive hair cell-like cells from embryonic and induced pluripotent stem cells. *Cell.* 2010;141(4):704-716.
doi: 10.1016/j.cell.2010.03.035
 25. Barker N, Van Es JH, Kuipers J, *et al.* Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature.* 2007;449(7165):1003-1007.
doi: 10.1038/nature06196
 26. Koo B-K, Clevers H. Stem cells marked by the R-spondin receptor LGR5. *Gastroenterology.* 2014;147(2):289-302.
doi: 10.1053/j.gastro.2014.05.007
 27. Chai R, Kuo B, Wang T, *et al.* Wnt signaling induces proliferation of sensory precursors in the postnatal mouse cochlea. *Proc Natl Acad Sci USA.* 2012;109(21):8167-8172.
doi: 10.1073/pnas.1202774109
 28. Shi F, Kempfle JS, Edge AS. Wnt-responsive Lgr5-expressing stem cells are hair cell progenitors in the cochlea. *J Neurosci.* 2012;32(28):9639-9648.
doi: 10.1523/JNEUROSCI.1064-12.2012
 29. Koehler KR, Mikosz AM, Molosh AI, Patel D, Hashino E. Generation of inner ear sensory epithelia from pluripotent stem cells in 3D culture. *Nature.* 2013;500(7461):217-221.
doi: 10.1038/nature12298
 30. McLean WJ, Yin X, Lu L, *et al.* Clonal expansion of Lgr5-positive cells from mammalian cochlea and high-purity generation of sensory hair cells. *Cell Rep.* 2017;18(8):1917-1929.
doi: 10.1016/j.celrep.2017.01.066
 31. Roccio M, Perny M, Ealy M, Widmer HR, Heller S, Senn P. Molecular characterization and prospective isolation of human fetal cochlear hair cell progenitors. *Nat Commun.* 2018;9(1):4027.
doi: 10.1038/s41467-018-06334-7
 32. Xia M, Ma J, Wu M, *et al.* Generation of innervated cochlear organoid recapitulates early development of auditory unit. *Stem Cell Rep.* 2023;18(1):319-336.
doi: 10.1016/j.stemcr.2022.11.024
 33. Moore ST, Nakamura T, Nie J, *et al.* Generating high-fidelity cochlear organoids from human pluripotent stem cells. *Cell Stem Cell.* 2023;30(7):950-961.e7.
doi: 10.1016/j.stem.2023.06.006
 34. Xia M, Wu M, Li Y, *et al.* Varying mechanical forces drive sensory epithelium formation. *Sci Adv.* 2023;9(44):eadf2664.
doi: 10.1126/sciadv.adf2664
 35. Matsui TK, Tsuru Y, Hasegawa K, Kuwako K-i. Vascularization of human brain organoids. *Stem Cells.* 2021;39(8):1017-1024.
doi: 10.1002/stem.3368
 36. Novosel EC, Kleinhans C, Kluger PJ. Vascularization is the key challenge in tissue engineering. *Adv Drug Deliv Rev.* 2011;63(4-5):300-311.
doi: 10.1016/j.addr.2011.03.004
 37. Nyberg S, Abbott NJ, Shi X, Steyger PS, Dabdoub A. Delivery of therapeutics to the inner ear: The challenge of the blood-labyrinth barrier. *Sci Transl Med.* 2019;11(482):eaao0935.
doi: 10.1126/scitranslmed.aao0935
 38. Wangemann P. Supporting sensory transduction: Cochlear fluid homeostasis and the endocochlear potential. *J Physiol.* 2006;576(Pt 1):11-21.
doi: 10.1113/jphysiol.2006.112888
 39. Hansen CE, Hollaus D, Kamermans A, de Vries HE.

Tension at the gate: Sensing mechanical forces at the blood-brain barrier in health and disease. *J Neuroinflammation*. 2024;21(1):325.

doi: 10.1186/s12974-024-03321-2

40. Poorna Pillutla SV, Kaur C, Roy TS, Jacob TG. Estimation of volume of stria vascularis and the length of its capillaries in the human cochlea. *J Microsc Ultrastruct*. 2019;7(3):117-123.
doi: 10.4103/JMAU.JMAU_12_19
41. Li Y, Sun X-Y, Zeng P-M, Luo Z-G. Neural Responses to Hypoxic Injury in a Vascularized Cerebral Organoid Model. *Neurosci Bull*. 2025;1-13.
doi: 10.1007/s12264-024-01346-2
42. Shaji M, Tamada A, Fujimoto K, Muguruma K, Karsten SL, Yokokawa R. Deciphering potential vascularization factors of on-chip co-cultured hiPSC-derived cerebral organoids. *Lab Chip*. 2024;24(4):680-696.
doi: 10.1039/d3lc00930k
43. Shi Y, Sun L, Wang M, *et al*. Vascularized human cortical organoids (vOrganoids) model cortical development in vivo. *PLoS Biol*. 2020;18(5):e3000705.
doi: 10.1371/journal.pbio.3000705
44. Salehi A, Rutz L, Ulbrich K, *et al*. Surface-modified gelatin hydrogel scaffolds with imprinted microgrooves: Physical characterization and study on endothelial cell interaction. *J Biomater Sci Polym Ed*. 2025;36(5):1-36.
doi: 10.1080/09205063.2025.2527912
45. Mastrullo V, Cathery W, Velliou E, Madeddu P, Campagnolo P. Angiogenesis in tissue engineering: As nature intended? *Front Bioeng Biotechnol*. 2020;8:188.
doi: 10.3389/fbioe.2020.00188
46. Walaas GA, Gopalakrishnan S, Bakke I, *et al*. Physiological hypoxia improves growth and functional differentiation of human intestinal epithelial organoids. *Front Immunol*. 2023;14:1095812.
doi: 10.3389/fimmu.2023.1095812
47. Kumano K, Nakahashi H, Louphrasitthiphon P, *et al*. Hypoxia at 3D organoid establishment selects essential subclones within heterogeneous pancreatic cancer. *Front Cell Dev Biol*. 2024;12:1327772.
doi: 10.3389/fcell.2024.1327772
48. Min S, Ko IK, Yoo JJ. State-of-the-art strategies for the vascularization of three-dimensional engineered organs. *Vasc Specialist Int*. 2019;35(2):77-89.
doi: 10.5758/vsi.2019.35.2.77
49. Almubarak S, Nethercott H, Freeberg M, *et al*. Tissue engineering strategies for promoting vascularized bone regeneration. *Bone*. 2016;83:197-209.
doi: 10.1016/j.bone.2015.11.011
50. Martino MM, Hubbell JA. The 12th–14th type III repeats of fibronectin function as a highly promiscuous growth factor-binding domain. *FASEB J*. 2010;24(12):4711-4721.
doi: 10.1096/fj.09-151282
51. Ebeid M, Huh S-H. FGF signaling: Diverse roles during cochlear development. *BMB Rep*. 2017;50(10):487-495.
doi: 10.5483/BMBRep.2017.50.10.164
52. Huh S-H, Warchol ME, Ornitz DM. Cochlear progenitor number is controlled through mesenchymal FGF receptor signaling. *eLife*. 2015;4:e05921.
doi: 10.7554/eLife.05921
53. Yang LM, Cheah KSE, Huh S-H, Ornitz DM. Sox2 and FGF20 interact to regulate organ of Corti hair cell and supporting cell development in a spatially-graded manner. *PLoS Genet*. 2019;15(7):e1008254.
doi: 10.1371/journal.pgen.1008254
54. Jacques BE, Montcouquiol ME, Layman EM, Lewandoski M, Kelley MW. Fgf8 induces pillar cell fate and regulates cellular patterning in the mammalian cochlea. *Development*. 2007;134(16):3021-3029.
doi: 10.1242/dev.02874
55. Zhang J, Hou Z, Wang X, *et al*. VEGFA165 gene therapy ameliorates blood-labyrinth barrier breakdown and hearing loss. *JCI Insight*. 2021;6(8):e143285.
doi: 10.1172/jci.insight.143285
56. Wan L, Lovett M, Warchol ME, Stone JS. Vascular endothelial growth factor is required for regeneration of auditory hair cells in the avian inner ear. *Hear Res*. 2020;385:107839.
doi: 10.1016/j.heares.2019.107839
57. Wang K, Lin R-Z, Hong X, *et al*. Robust differentiation of human pluripotent stem cells into endothelial cells via temporal modulation of ETV2 with modified mRNA. *Sci Adv*. 2020;6(30):eaba7606.
doi: 10.1126/sciadv.aba7606
58. Skylar-Scott MA, Huang JY, Lu A, *et al*. Orthogonally induced differentiation of stem cells for the programmatic patterning of vascularized organoids and bioprinted tissues. *Nat Biomed Eng*. 2022;6(4):449-462.
doi: 10.1038/s41551-022-00856-8
59. Palikuqi B, Nguyen D-HT, Li G, *et al*. Adaptable haemodynamic endothelial cells for organogenesis and tumorigenesis. *Nature*. 2020;585(7825):426-432.
doi: 10.1038/s41586-020-2712-z
60. Kim TM, Lee RH, Kim MS, Lewis CA, Park C. ETV2/ER71, the key factor leading the paths to vascular regeneration and angiogenic reprogramming. *Stem Cell Res Ther*. 2023;14(1):41.
doi: 10.1186/s13287-023-03267-x
61. Cakir B, Xiang Y, Tanaka Y, *et al*. Engineering of human brain organoids with a functional vascular-like system. *Nat*

- Methods*. 2019;16(11):1169-1175.
doi: 10.1038/s41592-019-0586-5
62. Cai H, Tian C, Chen L, *et al*. Vascular network-inspired diffusible scaffolds for engineering functional midbrain organoids. *Cell Stem Cell*. 2025;32(5):824-837.e5.
doi: 10.1016/j.stem.2025.02.010
 63. Nwokoye PN, Abilez OJ. Bioengineering methods for vascularizing organoids. *Cell Rep Methods*. 2024;4(6):100779.
doi: 10.1016/j.crmeth.2024.100779
 64. Han X, Saiding Q, Cai X, *et al*. Intelligent vascularized 3D/4D/5D/6D-printed tissue scaffolds. *Nano Micro Lett*. 2023;15(1):239.
doi: 10.1007/s40820-023-01187-2
 65. Arkenberg MR, Jafarkhani M, Lin CC, Hashino E. Chemically defined and dynamic click hydrogels support hair cell differentiation in human inner ear organoids. *Stem Cell Rep*. 2025;20(2):102386.
doi: 10.1016/j.stemcr.2024.12.001
 66. Park S, Kim YJ, Sharma H, *et al*. Graphene hybrid inner ear organoid with enhanced maturity. *Nano Lett*. 2023;23(12):5573-5580.
doi: 10.1021/acs.nanolett.3c00988
 67. Hu Y, Xing J, Zhang H, *et al*. Electroacoustic Responsive Cochlea-on-a-Chip. *Adv Mater*. 2024;36(24):2309002.
doi: 10.1002/adma.202309002
 68. Gavara N, Manoussaki D, Chadwick RS. Auditory mechanics of the tectorial membrane and the cochlear spiral. *Curr Opin Otolaryngol Head Neck Surg*. 2011;19(5):382-387.
doi: 10.1097/MOO.0b013e32834a5bc9
 69. Reichenbach T, Hudspeth AJ. The physics of hearing: Fluid mechanics and the active process of the inner ear. *Rep Prog Phys*. 2014;77(7):076601.
doi: 10.1088/0034-4885/77/7/076601
 70. Ji S, Almeida E, Guvendiren M. 3D bioprinting of complex channels within cell-laden hydrogels. *Acta Biomater*. 2019;95:214-224.
doi: 10.1016/j.actbio.2019.02.038
 71. Brassard JA, Nikolaev M, Hübscher T, Hofer M, Lutolf MP. Recapitulating macro-scale tissue self-organization through organoid bioprinting. *Nat Mater*. 2021;20(1):22-29.
doi: 10.1038/s41563-020-00803-5
 72. Homan KA, Gupta N, Kroll KT, *et al*. Flow-enhanced vascularization and maturation of kidney organoids in vitro. *Nat Methods*. 2019;16(3):255-262.
doi: 10.1038/s41592-019-0325-y
 73. Onesto MM, Kim J-i, Pasca SP. Assembloid models of cell-cell interaction to study tissue and disease biology. *Cell Stem Cell*. 2024;31(11):1563-1573.
doi: 10.1016/j.stem.2024.09.017
 74. Kang H-W, Lee SJ, Ko IK, Kengla C, Yoo JJ, Atala A. A 3D bioprinting system to produce human-scale tissue constructs with structural integrity. *Nat Biotechnol*. 2016;34(3):312-319.
doi: 10.1038/nbt.3413
 75. Yue K, Trujillo-de Santiago G, Alvarez MM, Tamayol A, Annabi N, Khademhosseini A. Synthesis, properties, and biomedical applications of gelatin methacryloyl (GelMA) hydrogels. *Biomaterials*. 2015;73:254-271.
doi: 10.1016/j.biomaterials.2015.08.045
 76. Miller JS, Stevens KR, Yang MT, *et al*. Rapid casting of patterned vascular networks for perfusable engineered three-dimensional tissues. *Nat Mater*. 2012;11(9):768-774.
doi: 10.1038/nmat3357
 77. Kolesky DB, Homan KA, Skylar-Scott MA, Lewis JA. Three-dimensional bioprinting of thick vascularized tissues. *Proc Natl Acad Sci USA*. 2016;113(12):3179-3184.
doi: 10.1073/pnas.1521342113
 78. Lee A, Hudson AR, Shiwardski DJ, *et al*. 3D bioprinting of collagen to rebuild components of the human heart. *Science*. 2019;365(6452):482-487.
doi: 10.1126/science.aav9051
 79. Skylar-Scott MA, Uzel SG, Nam LL, *et al*. Biomanufacturing of organ-specific tissues with high cellular density and embedded vascular channels. *Sci Adv*. 2019;5(9):eaaw2459.
doi: 10.1126/sciadv.aaw2459
 80. Murphy SV, Atala A. 3D bioprinting of tissues and organs. *Nat Biotechnol*. 2014;32(8):773-785.
doi: 10.1038/nbt.2958
 81. Ozbolat IT, Peng W, Ozbolat V. Application areas of 3D bioprinting. *Drug Discov Today*. 2016;21(8):1257-1271.
doi: 10.1016/j.drudis.2016.04.006
 82. Gungor-Ozkerim PS, Inci I, Zhang YS, Khademhosseini A, Dokmeci MR. Bioinks for 3D bioprinting: An overview. *Biomater Sci*. 2018;6(5):915-946.
doi: 10.1039/c7bm00765e
 83. Pedde RD, Mirani B, Navaei A, *et al*. Emerging biofabrication strategies for engineering complex tissue constructs. *Adv Mater*. 2017;29(19):1606061.
doi: 10.1002/adma.201606061
 84. Bernal PN, Bouwmeester M, Madrid-Wolff J, *et al*. Volumetric Bioprinting of Organoids and Optically Tuned Hydrogels to Build Liver-Like Metabolic Biofactories. *Adv Mater*. 2022;34(15):e2110054.
doi: 10.1002/adma.202110054
 85. Aisenbrey EA, Murphy WL. Synthetic alternatives to Matrigel. *Nat Rev Mater*. 2020;5(7):539-551.
doi: 10.1038/s41578-020-0199-8

86. Zhang Z, Gao S, Hu YN, *et al.* Ti₃C₂Tx MXene composite 3D hydrogel potentiates mTOR signaling to promote the generation of functional hair cells in cochlea organoids. *Adv Sci.* 2022;9(32):e2203557.
doi: 10.1002/advs.202203557
87. Wang Y, Li H, Zhang J, Chen M, Pan Y, Lou X. 3D Bioprinting Inner Ear Organ of Corti Organoids Induce Hair Cell Regeneration. *J Biomed Mater Res A.* 2025;113(3):e37892.
doi: 10.1002/jbm.a.37892
88. Zhang J, Liu L, Shen R, Lou X. Construction of organ of Corti organoid to study the effects of berberine sulfate on damaged auditory cells. *J Biomed Mater Res B Appl Biomater.* 2024;112(7):e35439.
doi: 10.1002/jbm.b.35439
89. McNamara LE, McMurray RJ, Biggs MJ, Kantawong F, Oreffo RO, Dalby MJ. Nanotopographical control of stem cell differentiation. *J Tissue Eng.* 2010;1(1):120623.
doi: 10.4061/2010/120623
90. Daley WP, Peters SB, Larsen M. Extracellular matrix dynamics in development and regenerative medicine. *J Cell Sci.* 2008;121(Pt 3):255-264.
doi: 10.1242/jcs.006064
91. Zhu F, Nie G, Liu C. Engineered biomaterials in stem cell-based regenerative medicine. *Life Med.* 2023;2(4):lnad027.
doi: 10.1093/lifemedi/lnad027
92. Kleinman HK, Martin GR. Matrigel: Basement membrane matrix with biological activity. *Semin Cancer Biol.* 2005;15(5):378-386.
doi: 10.1016/j.semcancer.2005.05.004
93. Nie J, Koehler KR, Hashino E. Directed differentiation of mouse embryonic stem cells into inner ear sensory epithelia in 3D culture. In: Bhargava N, editor. *Organ Regeneration: 3D Stem Cell Culture & Manipulation.* New York, NY, USA: Humana Press; 2017:67-83.
doi: 10.1007/978-1-4939-6949-4_6
94. Schneeberger K, Spee B, Costa P, Sachs N, Clevers H, Malda J. Converging biofabrication and organoid technologies: The next frontier in hepatic and intestinal tissue engineering? *Biofabrication.* 2017;9(1):013001.
doi: 10.1088/1758-5090/aa6121
95. Asim S, Tabish TA, Liaqat U, Ozbolat IT, Rizwan M. Advances in Gelatin Bioinks to Optimize Bioprinted Cell Functions. *Adv Healthc Mater.* 2023;12(17):e2203148.
doi: 10.1002/adhm.202203148
96. Sakai Y, Yamato R, Onuma M, Kikuta T, Watanabe M, Nakayama T. Non-antigenic and low allergic gelatin produced by specific digestion with an enzyme-coupled matrix. *Biol Pharm Bull.* 1998;21(4):330-334.
doi: 10.1248/bpb.21.330
97. Li N, Zhang Z, Zhang L, *et al.* Establishment of a cochlear organoid platform for remodeling the extracellular matrix. *ACS Nano.* 2025;19(29):26542-26561.
doi: 10.1021/acsnano.5c04897
98. Gerchman E, Hilfer SR, Brown JW. Involvement of extracellular matrix in the formation of the inner ear. *Dev Dyn.* 1995;202(4):421-432.
doi: 10.1002/aja.1002020411
99. Fareez UNM, Naqvi SAA, Mahmud M, Temirel M. Computational Fluid Dynamics (CFD) Analysis of Bioprinting. *Adv Healthc Mater.* 2024;13(20):e2400643.
doi: 10.1002/adhm.202400643
100. Goto-Silva L, Ayad NME, Herzog IL, *et al.* Computational fluid dynamic analysis of physical forces playing a role in brain organoid cultures in two different multiplex platforms. *BMC Dev Biol.* 2019;19(1).
doi: 10.1186/s12861-019-0183-y
101. Kanda GN, Tsuzuki T, Terada M, *et al.* Robotic search for optimal cell culture in regenerative medicine. *eLife.* 2022;11.
doi: 10.7554/eLife.77007
102. Abdul L, Xu J, Sotra A, *et al.* D-CryptO: Deep learning-based analysis of colon organoid morphology from brightfield images. *Lab Chip.* 2022;22(21):4118-4128.
doi: 10.1039/d2lc00596d
103. Ou HC, Santos F, Raible DW, Simon JA, Rubel EW. Drug screening for hearing loss: Using the zebrafish lateral line to screen for drugs that prevent and cause hearing loss. *Drug Discov Today.* 2010;15(7-8):265-271.
doi: 10.1016/j.drudis.2010.01.001
104. Dekkers JF, Wiegerinck CL, De Jonge HR, *et al.* A functional CFTR assay using primary cystic fibrosis intestinal organoids. *Nat Med.* 2013;19(7):939-945.
doi: 10.1038/nm.3201
105. Liu Q, Zhang L, Zhu M-S, Wan G. High-throughput screening on cochlear organoids identifies VEGFR-MEK-TGFB1 signaling promoting hair cell reprogramming. *Stem Cell Rep.* 2021;16(9):2257-2273.
doi: 10.1016/j.stemcr.2021.08.010
106. Romano DR, Hashino E, Nelson RF. Deafness-in-a-dish: Modeling hereditary deafness with inner ear organoids. *Hum Genet.* 2022;141(3):347-362.
doi: 10.1007/s00439-021-02325-9
107. Cong L, Ran FA, Cox D, *et al.* Multiplex genome engineering using CRISPR/Cas systems. *Science.* 2013;339(6121):819-823.
doi: 10.1126/science.1231143
108. Takahashi K, Tanabe K, Ohnuki M, *et al.* Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell.* 2007;131(5):861-872.

- doi: 10.1016/j.cell.2007.11.019
109. Zhong C, Chen Z, Luo X, *et al.* Barhl1 is required for the differentiation of inner ear hair cell-like cells from mouse embryonic stem cells. *Int J Biochem Cell Biol.* 2018;96:79-89.
doi: 10.1016/j.biocel.2018.01.013
 110. Tang P-C, Nie J, Lee J, *et al.* Defective Tmprss3-associated hair cell degeneration in inner ear organoids. *Stem Cell Rep.* 2019;13(1):147-162.
doi: 10.1016/j.stemcr.2019.05.014
 111. White PM, Doetzlhofer A, Lee YS, Groves AK, Segil N. Mammalian cochlear supporting cells can divide and trans-differentiate into hair cells. *Nature.* 2006;441(7096):984-987.
doi: 10.1038/nature04849
 112. Tao L, Yu HV, Llamas J, *et al.* Enhancer decommissioning imposes an epigenetic barrier to sensory hair cell regeneration. *Dev Cell.* 2021;56(17):2471-2485.e5.
doi: 10.1016/j.devcel.2021.07.003
 113. Sun Q, Tan F, Wang X, *et al.* AAV-regulated Serpine2 overexpression promotes hair cell regeneration. *Mol Ther Nucleic Acids.* 2024;35(4):102396.
doi: 10.1016/j.omtn.2024.102396
 114. Li X-J, Doetzlhofer A. LIN28B/let-7 control the ability of neonatal murine auditory supporting cells to generate hair cells through mTOR signaling. *Proc Natl Acad Sci USA.* 2020;117(36):22225-22236.
doi: 10.1073/pnas.2000417117
 115. Urciuolo A, Giobbe GG, Dong Y, *et al.* Hydrogel-in-hydrogel live bioprinting for guidance and control of organoids and organotypic cultures. *Nat Commun.* 2023;14(1):3128.
doi: 10.1038/s41467-023-37953-4
 116. DeForest CA, Tirrell DA. A photoreversible protein-patterning approach for guiding stem cell fate in three-dimensional gels. *Nat Mater.* 2015;14(5):523-531.
doi: 10.1038/nmat421901