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Pathophysiology of the cochlear intrastrial fluid-blood barrier (review)

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Abstract

The blood-labyrinth barrier (BLB) in the stria vascularis is a highly specialized capillary network that controls exchanges between blood and the intrastitial space in the cochlea. The barrier shields the inner ear from blood-born toxic substances and selectively passes ions, fluids, and nutrients to the cochlea, playing an essential role in the maintenance of cochlear homeostasis. Anatomically, the BLB is comprised of endothelial cells (ECs) in the strial microvasculature, elaborated tight and adherens junctions, pericytes (PCs), basement membrane (BM), and perivascular resident macrophage-like melanocytes (PVM/Ms), which together form a complex “cochlear-vascular unit” in the stria vascularis. Physical interactions between the ECs, PCs, and PVM/Ms, as well as signaling between the cells, is critical for controlling vascular permeability and providing a proper environment for hearing function. Breakdown of normal interactions between components of the BLB is seen in a wide range of pathological conditions, including genetic defects and conditions engendered by inflammation, loud sound trauma, and ageing. In this review, we will discuss prevailing views of the structure and function of the strial cochlear-vascular unit (also referred to as the “intrastrial fluid-blood barrier”). We will also discuss the disrupted homeostasis seen in a variety of hearing disorders. Therapeutic targeting of the strial barrier may offer opportunities for improvement of hearing health and amelioration of auditory disorders.

1. Introduction

The inner ear is a remarkably stable homeostatic system controlled by a range of regulatory mechanisms, including control over ion, fluid, and nutrient transport (active and passive) by the blood-labyrinth barrier (BLB). Precise regulation of substrate transport into and out of the inner ear is essential for maintaining the stable composition of inner ear fluids for hearing (Juhn et al., 1981; Juhn et al., 2001). Normal function of the stria vascularis (referred to as the “intrastrial fluid-blood barrier”) is critical for maintaining the ionic gradients and endocochlear potential (EP) required for sensory hair cell transduction (Hibino et al., 2010; Marcus et al., 1983; Quraishi et al., 2008; Salt et al., 1987; Spicer et al., 1996; Wangemann, 2002; Zhang et al., 2012). Dysfunction of the stria, including the intrastrial fluid-blood barrier, is considered to be one of the etiologies in a number of hearing

disorders, including autoimmune inner ear disease, noise-induced hearing loss, **age-related hearing loss**, and genetically linked hearing diseases (Lin et al., 1997; Neng et al., 2015; Shi, 2009; Yang et al., 2011; Zhang et al., 2015). Despite the importance of the intrastrial fluid-blood barrier, the physiology of the barrier is largely unknown. Recent progress has been made in detailing the structural complexity of the barrier points to the important role accessory cells, such as pericytes (PCs), perivascular resident macrophage-like melanocytes (PVM/Ms), and basement membrane (BM), play in the intrastrial fluid-blood barrier. This review provides a topical overview of intrastrial fluid-blood barrier structure and function as well as a synopsis of the dysfunction seen in particular barrier components in different hearing disorders. The review also introduces current methods used for studying the pathophysiology of the intrastrial fluid-blood barrier.

2. Major components and structure of the cochlear intrastrial fluid-blood barrier

The intrastrial fluid-blood barrier is a specialized capillary network characterized by a relative absence of endothelial cell (EC) fenestration (Juhn, 1988). Vascular ECs connected to each other by tight junctions (TJs) and an underlying BM form a diffusion barrier that prevents most blood-borne substances from entering the stria vascularis (Sakagami et al., 1999; Sakagami et al., 1987). Recent studies, however, have shown the intrastrial fluid-blood barrier to be more complex than the conventional view, as the barrier is shown to include a large number of PCs (Shi, 2009; Shi et al., 2008; Takeuchi et al., 2001) and PVM/Ms (Shi, 2010) in addition to ECs and the BM, as shown in Figure 1. Through close anatomical and chemical interactions, these cells monitor the state of ion, fluid, and nutrient flow into the stria vascularis from the circulation and trigger responses to changes in demand.

A population of around 1220 to 1300 PCs is found in the intrastrial fluid-blood barrier of the normal adult C57/6J mouse cochlea (Neng et al., 2015), as shown in Figure 2A. Extensively branched strial PCs tightly embrace the abluminal strial capillary wall and embed in the BM (Figure 2B). PCs are known to display a heterogeneous range of morphology, phenotype, and function in different tissues (Allt et al., 2001). PCs in the stria vascularis express platelet-derived growth factor receptor-β (PDGFR-β), desmin, neural/glial antigen 2 (NG2) and CD90 (Thy-1) (Shi et al., 2008). In particular, the strial PCs are notably rich in the structural protein desmin, an intermediate filament protein (Shi et al., 2008). The capillary network in the stria vascularis has a unique cellular architecture of polygonal loops. The rich desmin fibers in strial PCs are thought to give the loops mechanical strength and enhance the physical resilience of the vascular networks (Shi et al., 2008). By contrast, PCs of the spiral ligament express more contractile proteins such as α-SMA and tropomyosin (Franz et al., 2004; Shi et al., 2008). The PCs are known to be vital for vascular development, blood flow regulation, vascular integrity, angiogenesis, and tissue fibrogenesis (Allt et al., 2001; Balabanov et al., 1998; Betsholtz et al., 2005; Díaz-Flores et al., 1991; Dore-Duffy et al., 2006; Greenhalgh et al., 2013; Hall et al., 2014; Peppiatt et al., 2006; Quaegebeur et al., 2010; von Tell et al., 2006). We recently reported that strial PCs regulate TJ expression between ECs and are essential for intrastrial fluid-blood barrier integrity (Neng et al., 2013a). Strial PCs derived from neonatal day-10~15 mice dramatically promote spouting

angiogenesis in an *in vitro* cell-line-based 3D co-culture, as shown in Figure 6 (Neng et al., 2015). Studies from non-cochlear tissues show that PCs also contribute to BM formation by directly synthesizing type IV collagen, glycosaminoglycan, fibronectin, nidogen-1, **perlecan**, and laminin (Allt et al., 2001; Fisher, 2009; Shepro et al., 1993), and inhibit the activity of destabilizing matrix metalloproteinases (MMP), such as MMP-2 and MMP-9 (Zozulya et al., 2008). How strial PCs contribute to the composition and formation of strial BM has not been specifically studied.

A certain population of PVM/Ms reside in the intrastrial fluid-blood barrier of the normal adult C57/6J mouse cochlea (Neng et al., 2015). It is generally accepted the PVM/Ms originate from cochlear melanocytes derived from the neural crest which have migrated to the stria vascularis during development (Freyer et al., 2011; Steel et al., 1989; Steel et al., 1992; Wakaoka et al., 2013). The majority of PVM/Ms are capable of self-renewal and turn over within approximately a 10 month time frame from circulating blood cells (Shi, 2010). The PVM/Ms situate in close proximity beneath the subepithelial layer of marginal cells and are highly invested on the abluminal surface of capillaries with multiple thin membrane protrusions (Figure 3A-3C). PVM/Ms are a hybrid cell type, displaying characteristics of both macrophage and melanocyte (Zhang et al., 2012). Earlier studies have shown the strial PVM/Ms express a number of macrophage surface markers including F4/80, CD68, and CD11b as well as macrophagic scavenger receptor classes A (1) and B (1) (Shi, 2010). Later studies have also shown the PVM/Ms to exhibit melanocyte characteristics, including containing significant amounts of melanin and expression of melanocyte marker proteins such as glutathione S-transferase alpha 4 (Gstα4) and Kir 4.1, the latter being the fiduciary marker of intermediate cells (Zhang et al., 2012).

A recent study shows that physical contact and signal communication between ECs and PVM/Ms is necessary for controlling intrastrial fluid-blood barrier integrity (Zhang et al., 2012). PVM/Ms, as a type of melanocyte, produce melanin pigment in response to noxious factors in the local tissue environment (Soulas et al., 2009; Sulaimon et al., 2003). The melanin plays an essential immunological role in tissue homeostasis by buffering calcium, scavenging heavy metals, foreign protein, and lipids, and promoting antioxidant activity (Bush et al., 2007; Dräger, 1985; Murillo-Cuesta et al., 2010; Ohlemiller et al., 2009; Plonka et al., 2009; Slominski, 2009; Slominski et al., 2012). PVM/Ms, as tissue resident macrophages in the cochlea, may also have a role in immunological defense and repair (Cui et al., 2009; Ekdahl et al., 2009). For example, tissue resident macrophages can scavenge invading microorganisms and dead cells, and the macrophages act as immune or immuno-effector cells by producing superoxide anions, nitric oxide, and inflammatory cytokines during inflammation in non-cochlear tissue (Block et al., 2005; Block et al., 2007; Chéret et al., 2008; Mitrasinovic et al., 2002; Mitrasinovic et al., 2005; Nimmerjahn et al., 2005). Some studies have also reported that tissue resident macrophages are capable of differentiating into fibroblast/myofibroblast for repair of damaged tissue in other organs (Pilling et al., 2009; Pufe et al., 2008; Takagi et al., 2008).

The intrastrial fluid-blood barrier also consists of extensive BM. Major components of the BM are collagen IV, laminin, heparan sulphate proteoglycan (HSPG), entactin/nidogen, and fibronectin (Cosgrove et al., 1996; Gratton et al., 2002; Satoh et al., 1998). Beta 1 and alpha

1 integrin subunits, as well as usherin, are also found in the BM (Bhattacharya et al., 2002; Tsuprun et al., 2001). Electron microscopy studies of the chinchilla cochlea shows the BM uniquely organized, with different proteins in the BM interacting to form a homogeneous lamina densa (Tsuprun et al., 2001). At the ultrastructural level, the distribution of proteoglycans in the BM is heterogeneous and patterned. The strial BM is also found to be negatively charged (Suzuki et al., 1996). **Anionic** sites have been identified in the BM of the strial capillary wall in both mouse and guinea pig strains (Suzuki et al., 1996; Suzuki et al., 1997; Suzuki et al., 1991; Suzuki et al., 1995; Torihara et al., 1994).

3. Intrastrial fluid-blood barrier permeability

Information on the regulation of blood barrier permeability in the **stria vascularis** is very limited. Earlier studies have shown the strial blood barrier in immature animals is more permeable than in adult animals. For example, Suzuki et al., (1998) reported that the strial blood barrier in rats isn't well formed until ~14 days after birth.

Two major pathways including para-cellular and transcellular pathways have been proposed to **control** vascular permeability in the intrastrial blood barrier. The para-cellular pathway is related to the transient state of TJs between ECs. The trans-cellular pathway involves endocytotic activity and trans-endothelial channels. The major TJ proteins in the intrastrial fluid–blood barrier are occludin, claudins, zona occludens, and adherens junction proteins such as VE-cadherin (Kitajiri et al., 2004; Neng et al., 2013a; Yang et al., 2011). Recent experiments have shown that up-regulation of TJ and adherens junction protein improves barrier integrity (Zhang et al., 2012), while, conversely, down-regulation of TJ and adherens junction protein increases permeability (Neng et al., 2013a; Zhang et al., 2012). Transcellular permeability, on the other hand, is dependent on transporters and channels. The intrastrial fluid–blood barrier is found to be rich in transporters. For example, with mass spectrometry analysis of isolated strial capillaries, Yang et al., (2011) reported that about 40% of barrier proteins relate to transport activities. ATP1A1 is the most prevalent molecular transporter on a mass basis.

Permeability of the vascular barrier has also been shown to be related to basement membrane properties such as density and molecular conformation in other organs (Azzi et al., 2013; Qiu et al., 2012). However, how the change of BM structure in the intrastrial fluid-blood barrier influences the vascular permeability has not yet been studied.

4. Intrastrial fluid-blood barrier and hearing disorders

Recent experimental results from different laboratories demonstrate the potential link of dysfunction of the intrastrial fluid-blood barrier in different hearing disorders, including noise-induced hearing loss (Shi, 2009; Shi et al., 2003; Zhang et al., 2012), age-related hearing loss (Gratton et al., 1996; Gratton et al., 1997; Neng et al., 2015; Ohlemiller et al., 2009), autoimmune disease (Lin et al., 1997; Ruckenstein et al., 1999), genetic hearing disorders (Cohen-Salmon et al., 2007; Fujimura et al., 2005; Jabba et al., 2006; Kitamura et al., 1994), and inflammatory edema and hydrops (Hirose et al., 2014; Zhang et al., 2015). In addition, the barrier is also the point of entry for certain ototoxic drugs, such as cisplatin and

gentamicin, which permeate the cochlea through the blood barrier and damage hearing function (Dai et al., 2008; Laurell et al., 2000; Wang et al., 2009). Although these pathological conditions have common features such as changes in vascular permeability and selectivity of the intrastrial fluid-blood barrier, each of them has distinct characteristics.

Noise-induced hearing loss (NIHL)

Acoustic trauma damages sensory cells, neurons, and support cells as well as disrupts the microcirculation in the cochlea (Canlon, 1987; Canlon, 1988; Hultcrantz et al., 1987; Kamogashira et al., 2015; Kujawa et al., 2015; Liberman et al., 2015; Ohlemiller et al., 2007; Shi, 2009; Shi et al., 2007; Wang et al., 2002; Yoshida et al., 1999). Increased vascular permeability, reduced circulation (ischemia), aggregation of leukocytes, and injury to endothelial cells are frequently seen in loud sound exposed animals (Goldwyn et al., 1997; Hultcrantz et al., 1987; Lamm et al., 1999; Quirk et al., 1992; Scheibe et al., 1993; Seidman et al., 1999; Shi et al., 2007; Suzuki et al., 2002). Further studies have revealed structural and molecular changes **in the intrastrial fluid-blood barrier** after acoustic trauma, including decreased expression of tight- and adherens-junction proteins, loosened TJs between ECs, and increased vascular permeability (Yang et al., 2011; Zhang et al., 2013). PCs are particularly vulnerable to **acoustic trauma.** Upon exposure to loud sound, some PCs undergo changes. They develop irregularities in their processes and can migrate from their normal locations attached to endothelial cells, resulting in destabilization of the intrastrial fluid-blood barrier (Shi, 2009). However, the specific signals underlying these changes in cochlear PCs have not been identified. Acoustic trauma also causes a proportion of PVM/Ms to activate, as shown in Figure 4 A-C. The traumatized PVM/Ms produce less PEDF, leading to down-regulation of tight junction-associated proteins and vascular leakage (Zhang et al., 2013). The PEDF produced by normal PVM/Ms is essential for stabilizing the intrastrial fluid–blood barrier, as the PEDF regulates expression of tight junction-associated proteins such as ZO-1 and VE-cadherin (Zhang et al., 2012).

Age-related hearing loss

Capillary damage and regression in aged animals have long been observed (Gratton et al., 1996; Gratton et al., 1997; Neng et al., 2015; Ohlemiller et al., 2008; Ohlemiller et al., 2009; Prazma et al., 1990; Schulte et al., 1992). A degree of strial capillary loss is found in both aged C57/J (Neng et al., 2015) and genetically deficient NOD NON-H2nb1 mice (Ohlemiller et al., 2008). In a human temporal bone study, presbycusis patients showed atrophy of the stria vascularis (Sprinzl et al., 2010). Thickened basement membrane and increased immunoglobulin and laminin deposits are found in the aged strial capillaries (Gratton et al., 1996; Sakaguchi et al., 1997a; Sakaguchi et al., 1997b). For example, Thomopoulos et al., (1997) reported 65-85% of gerbils 33 months or older had strial capillaries with thickened basement membranes (BM), exceeding by several-fold that observed in young controls. Aged animals also exhibit a significant decrease in PC and PVM/M density, accompanied by marked morphological changes in all regions of the stria vascularis (Neng et al., 2015). For example, young C57/6BJ animals (<3 months) have an abundance of PCs with a flat and slender morphology, and are tightly associated with ECs. The PCs in older animals (>6 months) are less abundant and have a prominent round body in less physical contact with the endothelium, a morphology previously described as a sign of

PC migration (Pfister et al., 2008). At the ultrastructural level, the PCs from aged animals show a loss of cytoplasmic organelles, have a vacuolated appearance, and are detached from ECs (Neng et al., 2015). PVM/Ms are also dramatically different in aged animals. For example, in younger C57/6J mice, PVM/Ms typically exhibit long and branched processes, and are closely associated with strial capillaries, as shown in Figure 4H-I. However, in animals at age 6, 9, and 12 months some of the PVM/Ms show retraction of branching. At age 21 months, some of the PVM/Ms are flat and amoeboid-shaped, as shown in Figure 4J, and exhibit less physical contact with the capillaries. Concurrent with the morphological changes, biochemical markers on the PVM/Ms also undergo a significant change. Terminal galactopyranosyl groups are exposed on membrane surfaces in the older animals, detected by binding to the lectin GS-IB4, as shown in Figure 4. This is the hallmark of macrophage activation (Maddox et al., 1982; Neng et al., 2015).

Autoimmune hearing disorder

Autoimmune disease in the inner ear often causes progressive sensorineural hearing loss and sometimes presents with vestibular symptoms (Meniere's disease) (Fujioka et al., 2014; Goodall, 2015; Greco et al., 2012; Hughes et al., 1983; Kim et al., 2014; Sara et al., 2014). There is strong evidence showing strial capillaries may be a target of autoimmune disease (Ågrup et al., 2006; Goodall, 2015; Lin et al., 1997; Takahashi et al., 1988). For example, deposition of immune-complexes and direct attack of the endothelium by autoantibodies are common features in these hearing and vestibular disorders (Ågrup et al., 2006; Goodall, 2015; Lin et al., 1997; Takahashi et al., 1988). Research using a C3H/lpr autoimmune mouse model has demonstrated the primary defect in hearing is breakdown of strial blood barrier integrity, IgG deposition in strial capillaries, and thickening of the basement membrane (Lin et al., 1997; Trune et al., 1989; Wong et al., 1992; Young et al., 1988). In clinical studies, blood drawn from patients with autoimmune hearing disorders show high levels of anti-endothelial and anti-phospholipid antibodies, including anti-choline transporter-like protein 2 and anti-heat shock protein (HSP70) (Cadoni et al., 2002; Mijovic et al., 2013; Mouadeb et al., 2005; Nair et al., 2004; Ottaviani et al., 1999; Toubi et al., 2004; Yehudai et al., 2006). Restoration of strial vascular function may be effective in treating autoimmune hearing loss.

Genetic hearing loss

Genetic defects in intrastrial fluid-blood barrier components have been identified in several genetically linked hearing loss pathologies, including Norrie Disease, Alport syndrome, Nr3b2(−/−) and Light (Blt) mutant, white spotting (Ws) and Varitint-waddler-J (VaJ) mouse mutants, and **connexin 30** deficiency related hearing loss ((Rehm et al., 2002) (Cable et al., 1998; Cable et al., 1992; Cable et al., 1993; Chen et al., 2007; Cohen-Salmon et al., 2007; Fujimura et al., 2005; Gratton et al., 2005; Kitamura et al., 1994; Kruegel et al., 2013; Ruan et al., 2005; Zallocchi et al., 2013). Norrie Disease presents with profound sensorineural deafness. The primary cause of Norrie Disease is the strial avascularity associated with an *Ndp* gene defect (Rehm et al., 2002). Dominant white spotting [W/W(v) and W(v)/W(v)] mice are well-known mutants with profound sensorineural hearing loss (Cable et al., 1992; Fujimura et al., 2005). The mutants lack strial intermediate cells. The mice also display a marked thickening of the basement membrane on strial capillaries as well as IgG deposits, similar to that found in aged animals and animals with autoimmune sensorineural hearing

loss (Cable et al., 1992; Fujimoto, 1995; Kitamura et al., 1994). Light (Blt) mutant mice lack melanocytes, resulting in strial atrophy and loss of the EP (Cable et al., 1993). Alport syndrome with high-frequency sensorineural hearing loss results from mutations in genes coding for collagen alpha3, alpha4, or alpha5 (Gratton et al., 2005; Kruegel et al., 2013; Zallocchi et al., 2013), and the syndrome exhibits as a thickened capillary basement membrane in the stria. **Hearing loss in Nr3b2** mutant mice is associated with reduced density of strial capillaries (Chen et al., 2007) and deficiency in connexin 30, both of which disrupt the intrastrial fluid-blood barrier (Cohen-Salmon et al., 2007). Recent research shows Spinster homolog 2 (Spns2)-deficient mice to rapidly lose auditory sensitivity and EP at 2 to 3 weeks of age. The pathology presents with significant structural changes in strial capillary and marginal cell boundaries (Chen et al., 2014).

Inflammation

Inflammatory factor-induced hearing disorders are hypothesized to be associated with disrupted vascular integrity in the stria vascularis and disturbed endolymph ion homeostasis (Hilger, 1952; Trune et al., 2012). In support of the hypothesis, Zhang et al., (2015) recently showed lipopolysaccharide-induced middle ear inflammation to disrupt the cochlear intrastrial fluid-blood barrier by down-regulating tight junction protein expression. Correspondingly, Hirose et al., (2014) demonstrated that lipopolysaccharide increases entry of serum fluorescein into the perilymph via the blood barrier **(Hirose et al., 2014)**. A study by Quintanilla-Diek, et al. (2013) showed lipopolysaccharide-induced inflammation to increase cytokine levels in the murine cochlea. Action by cytokines may be one of the causes for the increased permeability of the blood barrier. Previous studies have also shown that viral or bacterial infection induces anti-endothelial (anti-phospholipid) antibody attack on glycocalyx components in the barrier (Blank et al., 2007; Blank et al., 2004). These results indicate systemic or local inflammatory events can perturb the normal function of the blood barrier, resulting in homeostatic imbalance and hearing loss.

Intrastrial fluid–blood barrier as an ototoxic drug target

A variety of drugs, including antibacterial aminoglycoside antibiotics such as gentamicin and amikacin, anticancer agents such as cisplatin, carboplatin, nedaplatin, and oxaliplatin as well as loop diuretics such as furosemide, have side effects that damage the sense of hearing or balance in humans and animals (Ding et al., 2012; Kamogashira et al., 2015; Karasawa et al., 2011; Oishi et al., 2012; Rybak et al., 2007; Schacht et al., 2012). Recent research shows the intrastrial fluid–blood barrier might be a **main port of entry** for certain ototoxic drugs from the blood into cochlear fluids (Adamson, 2009; Dai et al., 2008; Laurell et al., 2000; Wang et al., 2009). This is further evidenced by the enhanced drug uptake and significantly increased hearing damage when the barrier is disrupted by diuretics or noise exposure (Ding et al., 2007; Li et al., 2015). Vasoactive peptides also augment cochlear uptake of ototoxic drugs such as gentamicin (Koo et al., 2011). The pathway of drug uptake from the stria vascularis is found to involve transporter systems as well as channels, including transient receptor potential cation channel subfamily V member 4 (TRPV4) in strial capillaries (Ishibashi et al., 2009; Karasawa et al., 2008). There is less evidence for paracellular transport of the drugs through the barrier (Laurell et al., 1997). Extensive experimental research shows that some ototoxic drugs, including cisplatin, cause structural damage to the

stria vascularis (Campbell et al., 1999; Cardinaal et al., 2000; Kohn et al., 1991; Meech et al., 1998).

5. Experimental models for study of the intrastrial fluid-blood barrier

Direct measurement of **the** strial blood barrier function is challenging and techniques for assessing blood barrier function are still under development. However, recently developed *in vitro* and *in vivo* experimental methods are providing an opportunity for more intensive study of the intrastrial fluid-blood barrier than was previously possible. In particular, recently developed *in vitro* cell line-based models enable direct investigation of cell-cell interactions, which provides a window for better understanding of how intrastrial fluid–blood barrier integrity is maintained.

In vitro cell line based blood barrier models have been widely used in functional studies of the blood-brain barrier and blood-retina barrier for decades (Duport et al., 1998; Lai et al., 2005). In recent work on the inner ear, Neng et al., (2013b) demonstrated use of three different *in vitro* blood barrier 2D models to directly study the specific role of cochlear EC, PC, and PVM/M signaling on blood-labyrinth barrier integrity. The models included a co-cultivated Transwell model, shown in Figure 6a (Model I), conditioned medium co-culture model, shown in Figure 6b (Model II), and CytoVu/SiMPore thin membrane co-culture model, shown in Figure 6c (Model III). The purpose of the study at hand will determine which *in vitro* blood barrier model is most suitable. For example, the co-cultivated Transwell and conditioned medium co-culture models enable investigators to explore the effect of signaling in paracrine / juxtacrine pathways between vascular cells on TJ formation. The models are also useful for testing drug candidates (studies of drug permeability, toxicity, interaction with efflux transporters). Compared with models I and II, the CytoVu/SiMPore thin membrane co-culture model enables more intensive study of the vascular direct cell-cell interactions which underlie formation of the barrier.

In addition to cell culture–based 2D *in vitro* blood barrier models, newly developed cell culture–based 3D matrigel models provide an additional tool for understanding how individual vascular cell interactions affect the properties of the vessel. For example with this experimental model, , Neng et al., (2015) showed PVM/Ms **play** an essential role in stabilizing the artificial capillary networks formed in endothelial cell cultures (Figure 6). Likewise, the strial PCs were shown to play a role in the sprouting of new blood vessels characteristic of angiogenesis, as shown in Figure 6.

In vitro cell culture-based models are useful tools for determining the interaction between vascular blood barrier cells and testing potential drug candidates, although care should be exercised in selecting the model. Some models are better suited to the task than others and caveats apply to all. In addition, factors such as formulation of the medium and cell passage number can result in significant variation. It should also be kept in mind the information gained from the *in vitro* model may not directly reflect the functionality of the blood barrier under physiological conditions. The findings will always need to be validated in an *in vivo* animal model.

Newly developed *in vivo* mouse-based models, including intra-vital fluorescence microscopy and enhanced MRI techniques, give the larger picture of blood barrier function under relatively intact physiological conditions. The recently developed thin or open vessel-window approach, used in combination with fluorescence intra-vital microscopy, can be used to study barrier physiology in the cochlear lateral wall under normal and pathological conditions (Shi et al., 2014). The open vessel-window approach, if used in conjunction with a high spatial and temporal resolution imaging system and fluorescent tracers, can provide sufficient resolution for determination of vascular permeability and cell migration. The approach is also useful for examining changes in cell morphology and other pathophysiological parameters in mouse models. For example, genetic NG_2 labeled PCs can be observed in NG_2DsRedBAC transgenic mice in the open window preparation (as shown in Figure 7D) and PC contractility in response to agents such as $CaCl_2$ in lateral wall capillaries can also be studied (Shi et al., 2014). The open vessel-window approach has also proved extremely useful for tracing the extravasation of GFP^+-labeled cells (or other inflammatory cells) from the intrastrial blood barrier (shown in Figure 7E) and assessing vascular permeability under different conditions (Shi et al., 2014). However, experiments with the open vessel-window approach are challenging. Subtle animal movements, such as those from animal breathing, arterial pulse, pulsatile blood flow, and CSF communication with perilymph, cause translation of the imaging location, making correlation and interpretation of the results difficult. Minimization of animal motion and stabilization of image focus are critical for obtaining useable data.

Alternatively, a thinned vessel-window approach, recently adopted from the thin cranial window (Drew et al., 2010), minimizes disruption of the homeostatic balance in the lateral wall and enables study of barrier function under relatively intact physiological conditions. The thin vessel-window approach allows visualization of vascular structures in the lateral wall for longer periods of time. For example, a successful thin vessel-window remains clear for about a week (Shi et al., 2014). However, the main drawback with the thin window preparation is the increased optical scattering (relative to the open window preparation) and the bone auto-fluorescence which increases the background signal in the image. The increased background limits the image resolution. Another challenge is that an exceptionally steady hand with fine surgery skills (under the microscope) are needed to obtain a smooth bone surface which minimizes light scattering.

Magnetic resonance imaging (MRI)

Recent developments in MRI offer exciting opportunities for structural, functional, and metabolic investigation in an intact, living cochlea. In particular, dynamic contrast enhanced (DCE) MRI using gadolidium-chelate (GdC) or **gadolidium** based contrast agents (GBCA) is increasingly being used to assess changes in vascular permeability (Le Floc'h et al., 2014; Walton et al., 2015). Intense noise, inflammation, and immune reactions disrupt the blood-barrier and cause leakage of GdC. Using contrast enhanced-MRI and GBCA, lipopolysaccharide (LPS) induced vascular leakage can be assessed in guinea pig (Le Floc'h et al., 2014; Walton et al., 2015). In humans, the technique provides an opportunity to visualize the different cochlear compartments and directly evaluate the integrity of inner ear barriers. For example, using three-dimensional fluid attenuated inversion recovery (3D-

FLAIR3D-FLAIR) MRI, Tanigawa et al., (2010) found changes in the composition of the inner ear fluid in patients with low-tone sudden deafness. MRI also holds considerable potential for clinical assessment of labyrinth disease (Manfrè et al., 1999) and Meniere's disease (Pyykkö et al., 2010; Zou et al., 2009).

Summary

The intrastrial fluid-blood barrier comprises a microvascular endothelium closely associated with a substantial population of accessory cells (PCs and PVM/Ms) and a specific matrix of extracellular BM proteins that, together, constitute a unique “cochlear vascular unit.” A functional intrastrial fluid-blood barrier is critical for maintaining solute and ion homeostasis in the inner ear and preventing the influx of toxic substances in the stria vascularis. Disruption of the intrastrial fluid-blood barrier is considered to be involved in various clinical hearing disorders, including autoimmune inner ear disease, Meniere's disease, drug-induced hearing loss, noise-induced hearing loss, sudden deafness, and genetically-linked hearing dysfunction. Despite its central importance, the physiology of the intrastrial fluid-blood barrier in hearing health and disease remains poorly understood. The current lack of knowledge is limiting development of effective drug therapies for blood barrier dysfunction-related hearing loss. A better understanding of intrastrial fluid blood barrier pathophysiology is the foundation needed for developing new medical interventions treating blood barrier related hearing loss and balance disorders.

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Highlights

The blood-labyrinth-barrier (BLB) in the stria vascularis (“intra-strial fluid-blood barrier”**)** is comprised of endothelial cells in the strial microvasculature, elaborated tight and adherens junctions, basement membrane, pericytes, and perivascular resident macrophage-like melanocytes, which together form a complex “cochlear-vascular unit” in the stria vascularis.

A functional intra-strial fluid-blood barrier is critical for maintaining solute and ion homeostasis and preventing the influx of toxic substances in the inner ear.

Disruption of intra-strial fluid-blood barrier is associated with many clinical hearing disorders, including autoimmune inner ear disease, drug-induced hearing loss, noise-induced hearing loss, and genetically-linked hearing dysfunction. Therapeutic targeting of BLB function may lead to improved hearing health and the amelioration of hearing loss.

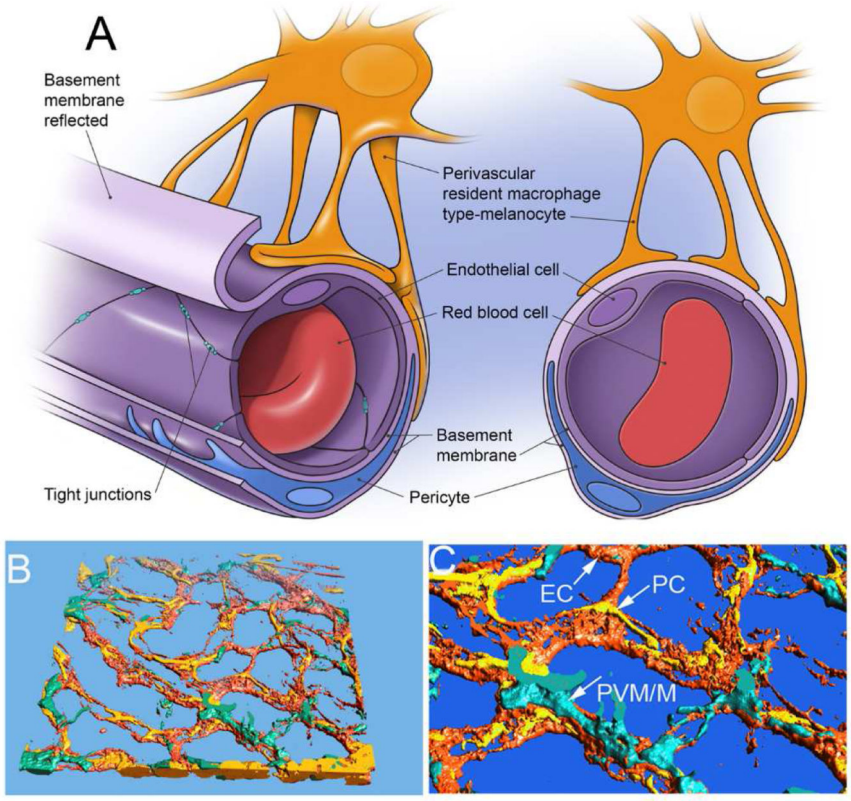

Figure 1.

(**A**) The illustration of a cochlear micro-vessel in cross-section shows the major components of the intrastrial fluid-blood barrier. The vessel lumen comprises ECs connected by TJs. ECs are ensheathed by a dense basement membrane shared with PCs. PVM/M end-feet cover a large portion of the capillary surface. (**B**) & (**C**) The reconstructed confocal image of the intrastrial fluid-blood barrier highlights the morphological complexity of interactions between ECs, PCs, and PVM/Ms. The PVM/Ms are immunolabeled for F4/80, PCs for desmin, and ECs with fluorescent DiI.

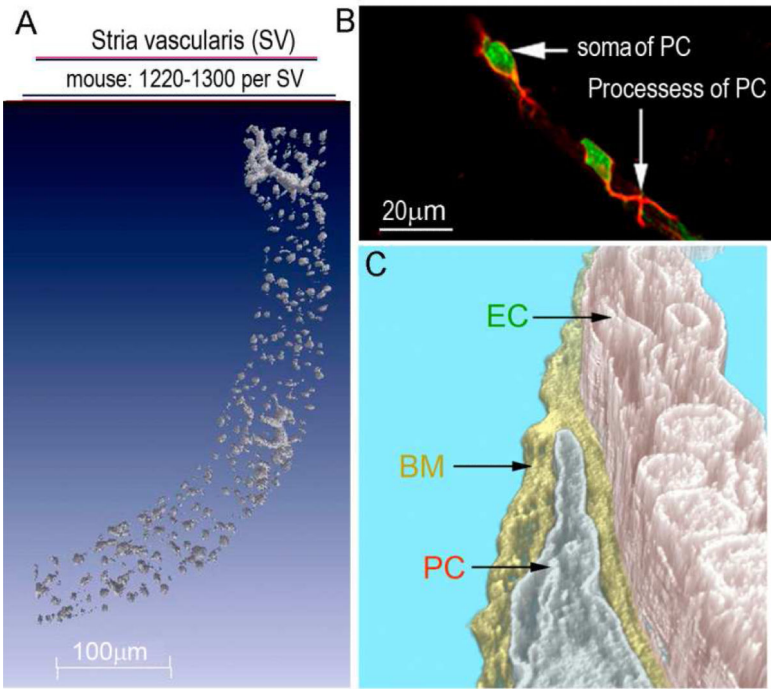

Figure 2.

(**A**) The super-resolution image shows the high density of strial PCs (labeled with NG2, gray) in the mouse SV (~1220-1300 PCs per SV). (**B**) The confocal projection shows the PC soma (stained with DAF-2DA, short arrow, green) and primary processes (labeled with desmin, long arrow, red). Two PCs are shown, each having a characteristic of a “bump on a log” shape, situated on the outer wall of a strial vessel (**PC**: pericyte, **PVM/M**: perivascular resident macrophage, **V/SV**: vessels of the stria vascularis, **V/SL**: vessels of the spiral ligament). (**C**) TEM tomography shows the cochlear PCs are embedded in the basement membrane (BM) and are closely associated with ECs. TEM tomography enables detection of the interactions between PCs, ECs, and the BM at high resolution (**BM**: basement membrane, **EC**: endothelial cell).

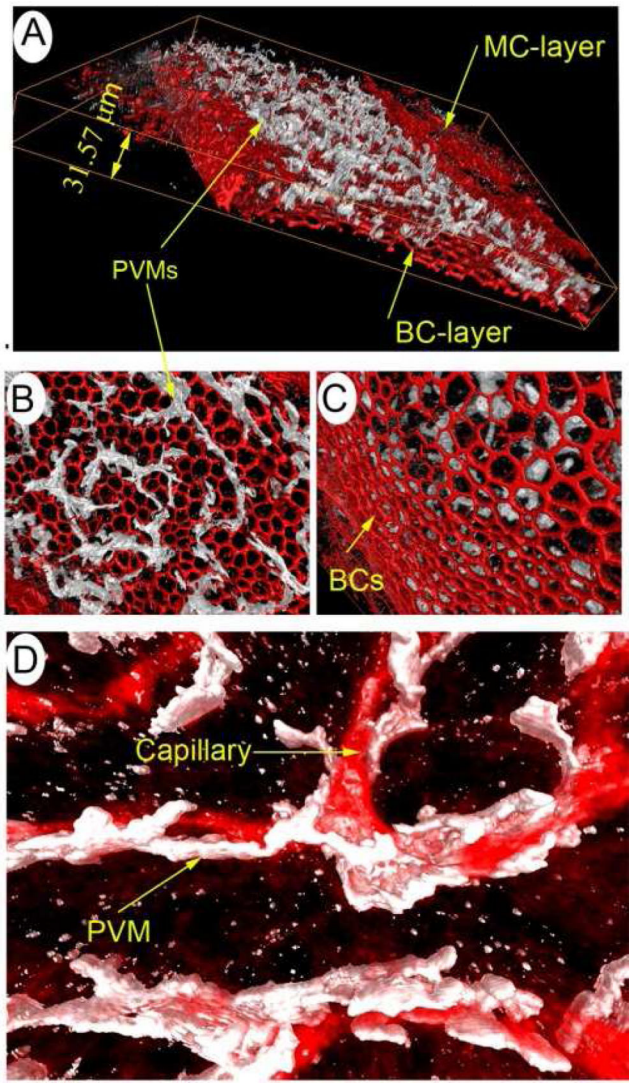

Figure 3.

PVM/Ms in the stria vascularis interface with capillaries. (**A**) PVM/Ms in the 3-D reconstruction are immunohistochemically labeled with antibody for F4/80 (white), the cytoskeleton labeled with Alexa Fluor® 568 phalloidin (red). The ramified processes of PVM/Ms are sandwiched between marginal and basal cell layers of the stria vascularis. (**B**) The 3-D reconstruction shows PVMs are situated in or under subepithelial marginal cells and have no basal cell contacts (**C**). (**D**) The ramified processes of PVM/Ms interface with the endothelial tube. Capillaries have been labeled with an antibody for IgG.

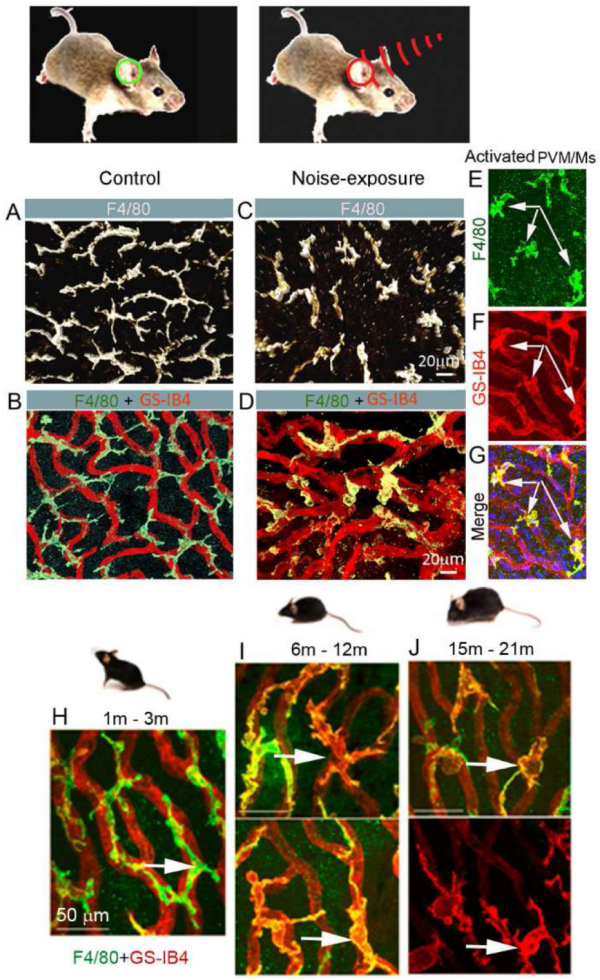

Figure 4.

Noise exposure activates PVM/Ms. (**A**) & (**B**) Confocal images show the morphology of PVM/Ms on strial capillaries labeled with GS-IB4 (red) in a control animal. (**C**) & (**D**) Activated PVM/Ms in noise-exposed animals show reduced branching and withdrawal of ramifications, and display less physical contact with capillaries. (**E**–**G**) Double-labeling of whole-mounted stria vascularis shows activated PVM/Ms are positive for GS-IB4. (**H**–**J**) show PVM/M activation in C57/6J mice at age 6m (**I**), with progressively more activation by age 15-21m.

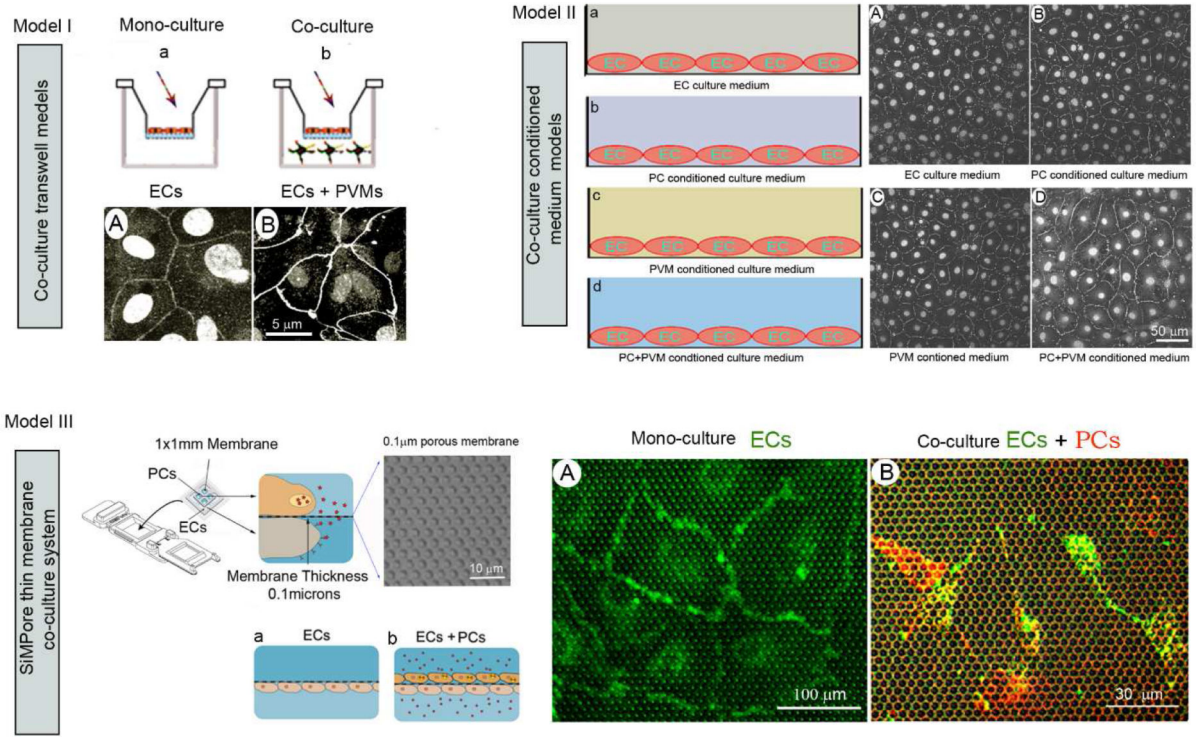

Figure 5.

The schematic illustrates several variations of the *in vitro* blood-labyrinth barrier model. (**a**) Model I shows a schematic of cells co-cultivated on a Transwell layer. Images of an EC monolayer labeled with antibody for ZO-1 are shown in both a mono-culture and co-culture setup. (**b**) Model II shows an EC monolayer labeled with antibody for ZO-1 and treated with different conditioning growth media. (**c**) Model III is a CytoVu/SiMPore thin membrane co-culture system. (**d**) The accompanying confocal fluorescence images show direct visualization of BSI-B4-labeled ECs on the thin membrane (left) and a population of PE-PDGFRβ–labeled PCs to one side of the thin membrane and FITC-BSI-B4–labeled ECs on the other (right).

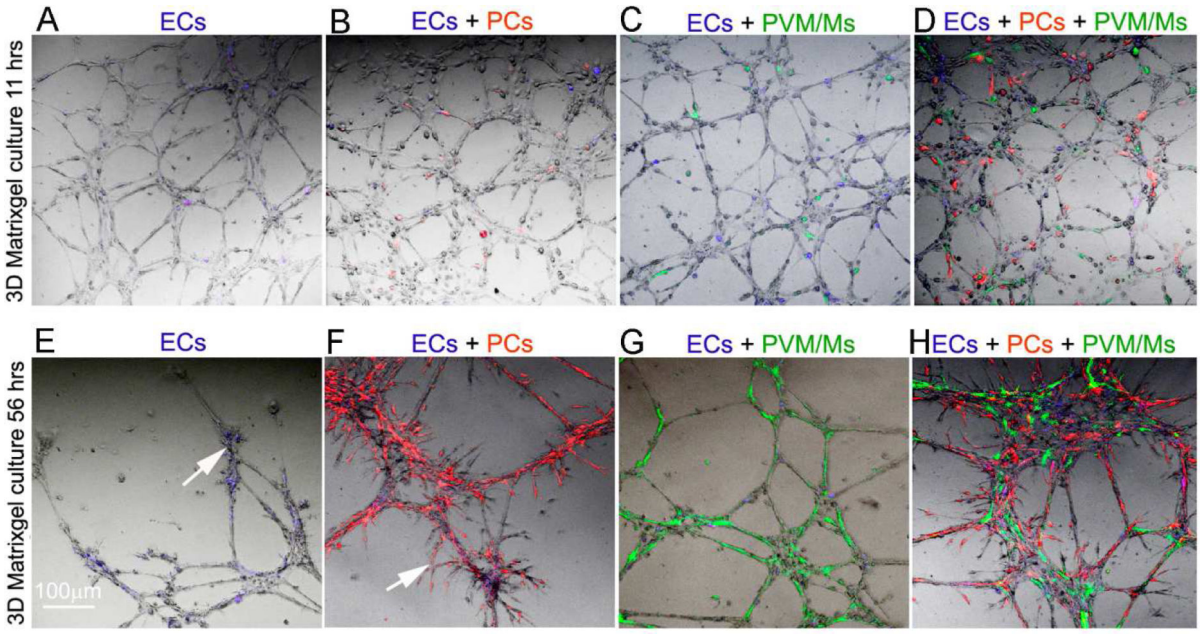

Figure 6.

Mono-culture of ECs and co-culture of ECs + PCs, ECs + PVM/ Ms, and ECs + PCs + PVM/Ms at various times in a three-dimensional (3-D) matrix gel. (**A**–**H**) Representative confocal images of a mono-culture of ECs and co-culture of ECs +PCs, ECs + PVM/Ms, and ECs + PCs + PVM/Ms at 11 h and 56 h in a matrix gel. No angiogenesis or EC tube regression is seen at 11 h in culture (**A**–**D**). Significant angiogenesis and EC tube formation is seen at 56 h (**E**–**H**). (**E**) Sparse branched networks and sprouting angiogenesis are seen in the EC alone group at 56 h (arrow). PCs promote sprouting angiogenesis of the tube structures (arrow). (**G**) PVM/Ms significantly delay regression of EC-formed capillary-like tubes. (**H**) Both PCs and PVM/Ms are required for capillary stability and angiogenesis. The PCs and PVM/Ms cooperate to promote sprouting angiogenesis.

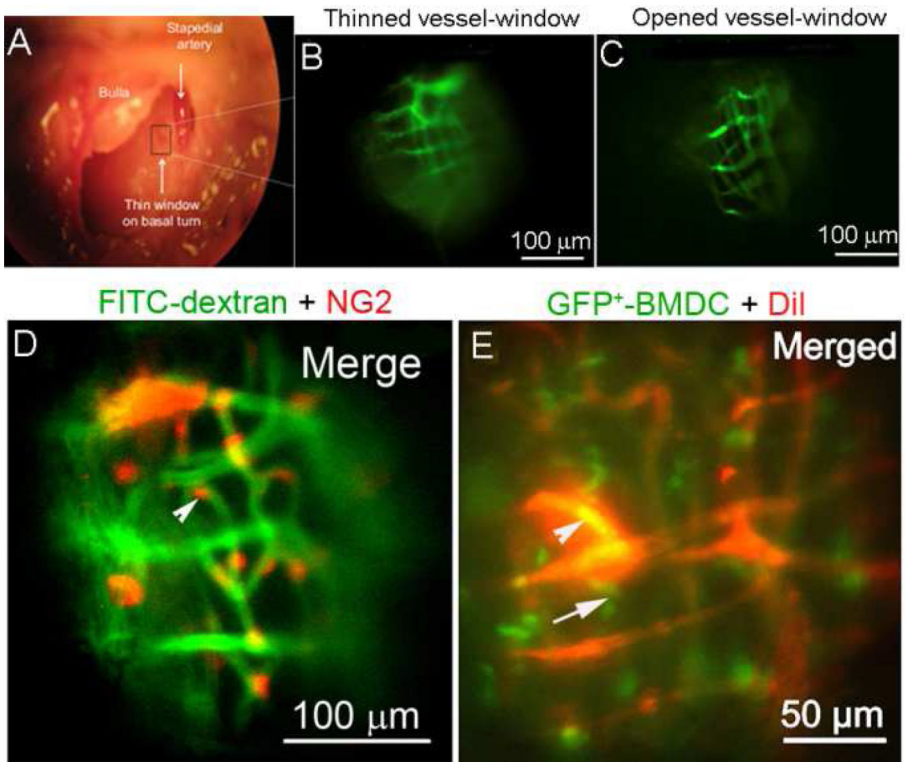

Figure 7.

(**A**) The surgical view shows an opened bulla and location of a vessel-window on the basal turn of a murine cochlea (black rectangle in panel **A**). Cochlear blood vessels are visualized with intravenously administered FITC-dextran. (**B**) & (**C**) compare *in vivo* images of strial blood vessels though a thin vessel-window and open vessel-window. (**D**) An open vessel-window preparation was used to visualize PCs and determine PC contractility using a NG_2 DsRed labeled-PC **transgenic** cell mouse model. (**E**) Migrated GFP^+-bone marrow cells are shown extravasated through blood vessels (red, labeled by DiI). The arrowhead alone points to a GFP^+ bone marrow cell within the vessel, the arrow to a GFP^+ bone marrow cell outside the vessel.