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A novel method for measuring hydraulic conductivity at the human blood-nerve barrier *in vitro*

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Abstract

Microvascular barrier permeability to water is an essential biophysical property required for the homeostatic maintenance of unique tissue microenvironments. This is of particular importance in peripheral nerves where strict control of ionic concentrations is needed for axonal signal transduction. Previous studies have associated inflammation, trauma, toxin exposure and metabolic disease with increases in water influx and hydrostatic pressure in peripheral nerves with resultant endoneurial edema that may impair axonal function. The regulation of water permeability across endoneurial microvessels that form the blood-nerve barrier (BNB) is poorly understood. Variations exist in apparatus and methods used to measure hydraulic conductivity. The objective of the study was to develop a simplified hydraulic conductivity system using commercially available components to evaluate the BNB. We determined the mean hydraulic conductivity of cultured confluent primary and immortalized human endoneurial endothelial cell layers as 2.00×10^{-7} and 2.17×10^{-7} cm/s/cm H₂O respectively, consistent with restrictive microvascular endothelial cells *in vitro*. We also determined the mean hydraulic conductivity of immortalized human brain microvascular endothelial cell layers, a commonly used blood-brain barrier (BBB) cell line, as 0.20×10^{-7} cm/s/cm H₂O, implying a mean 10-fold higher resistance to transendothelial water flux in the brain compared to peripheral nerves. To our knowledge, this is the first reported measurement of human BNB and BBB hydraulic conductivities. This model represents an important tool to further characterize the human BNB and deduce the molecular determinants and signaling mechanisms responsible for BNB hydraulic conductivity in normal and disease states *in vitro*.

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POTENTIAL CONFLICTS OF INTEREST

E.S.H. and S.D. have nothing to disclose.

Keywords

Blood-brain barrier; blood-nerve barrier; bubble tracker; diffusion chamber; endoneurial endothelial cells; hydraulic conductivity; peripheral nerves; transendothelial water flux

INTRODUCTION

Tight regulation of ion, metabolite, protein, and water concentrations within peripheral nerves is critical to maintaining the internal microenvironment needed for axonal signal transmission (Greathouse et al., 2016; Mizisin and Weerasuriya, 2011; Olsson, 1990). The blood supply to human nerves is derived from extrinsic arteries that form the vasa nervosum. These vessels subsequently branch to form an anastomosis of epineurial macrovessels and perineurial microvessels. The latter penetrate the innermost perineurium layer to form endoneurial microvessels. In contrast to the highly permeable epineurial blood vessels that lack tight junctions and are fenestrated, the endoneurial microvasculature lack fenestrations and consist of tight junction-forming endothelial cells. These cells share a basement membrane with pericytes that form a non-continuous surrounding layer along the endothelial abluminal border (Reina et al., 2000; Reina et al., 2003). As a consequence, endoneurial microvessels form the BNB. Current knowledge on the molecular and biophysical characteristics of the human BNB *in vitro*, including measures of transendothelial electrical resistance, solute permeability to small and large molecules, and response to cytokine and mitogen stimulus has been recently summarized (Ubogu, 2013).

The BNB regulates paracellular and transcellular transport crucial for peripheral nerve homeostasis. For example, the BNB maintains endoneurial albumin concentrations significantly lower than that of plasma. Under normal physiological conditions, the resulting oncotic pressure gradient opposes endoneurial edema by pulling water into microvessel lumens (Mizisin and Weerasuriya, 2011). However, elevated endoneurial albumin concentrations have been described in human diabetic neuropathy, postulated to occur as a consequence of BNB failure (Poduslo et al., 1988). Endoneurial compartment pressure in rat peripheral nerves has been reported as 1.2 ± 0.7 mmHg (1.6 ± 0.95 cm H₂O) (Low et al., 1977) and 2.0 ± 1.0 cm H₂O (Myers et al., 1978). This positive hydrostatic pressure is likely due to the lack of draining endoneurial lymphatic vessels (Sunderland, 1978). In contrast, most other tissues, including the surrounding epineurium, are drained by lymphatic vessels and thus under a negative hydrostatic pressure. The resulting positive hydrostatic gradient creates a physiological barrier that is critical for homeostatic maintenance by impeding potential diffusion of water from the surrounding epineurium. This consequentially establishes endoneurial microvessels as the primary path of water entry into the endoneurium. The BNB, in regulating water influx from or efflux to the blood circulation is essential to maintaining a constant endoneurial hydrostatic pressure.

Endoneurial edema is commonly observed in acute and chronic inflammatory neuropathies (Olsson, 1990; Ubogu, 2015), trauma due to nerve compression (Werner et al., 1983), reperfusion nerve injury (Nukada and McMorran, 1994), toxic insult such as lead poisoning (Myers et al., 1980), and diabetic neuropathy (Poduslo et al., 1988). These studies highlight

the association of specific disease states that may cause cytotoxic edema or elevated hydrostatic pressure within the endoneurium with a suboptimal response of the BNB to maintain endoneurial water flux necessary for peripheral nerve homeostasis. This deficit possibly contributes to the observed peripheral nerve dysfunction in these disorders. Hydraulic conductivity, a measure of fluid flux (typically water) across a barrier in response to an external stimulus such as a hydrostatic gradient, is an essential biophysical property of the epithelium and endothelium (Jacob and Chappell, 2013; Malik et al., 1989; Tarbell, 2010). The hydraulic conductivity of the BNB is currently unknown, and as a consequence, the essential molecular determinants and signaling mechanisms of transendothelial water flux into the peripheral nerve endoneurium in health and in disease are undetermined.

Significant variations exist in the apparatus and approach used to measure hydraulic conductivity, with several investigator-built or modified components, including software that are not commercially available. We developed a hydraulic conductivity system based on a commercially available transwell diffusion chamber model attached to a simplified customized digital bubble tracker device in order to measure water flux across the human BNB *in vitro*. We compared data to a restrictive human blood-brain barrier cell line and dermal fibroblasts that are relatively impervious to water but do not form tight junctions. This technical report serves as a preamble to future mechanistic studies on the regulation of this important biophysical process at the BNB relevant to physiological and pathophysiological conditions.

MATERIALS AND METHODS

Apparatus Assembly

To deduce hydraulic conductivity, the displacement of fluid across a semi-permeable membrane in a closed system without leaks following application of a hydrostatic pressure gradient is needed. Linear displacement of a bubble in constant diameter glass tube within this closed system directly measures fluid flux. As a consequence, a “bubble tracker” apparatus was constructed utilizing several components as depicted in Figure 1. These components included a custom machined aluminum breadboard used to assemble a slotted plate for holding six 20.3 cm long trubore[®] precision glass tubes (1.93 mm internal diameter; Ace Glass, Vineland, NJ), a camera mount, and a custom 6-channel hydrostatic pressure reservoir. 12 mm Snapwell cell culture inserts with 0.4 μ m pore polyester membranes (Corning Costar; Kennebunk, ME) were placed in modified EasyMount Ussing diffusion chambers housed in a 6-Channel EasyMount stand (Physiologic Instruments, Inc.; San Diego, CA).

The EasyMount stand was connected to a circulating water bath calibrated to maintain insert chamber temperatures at 37°C. The components for each chamber were connected using Masterflex BioPharm Plus platinum-cured silicone tubing (1.5875 mm internal diameter) and buffer flow at critical junctures was controlled using 3-way stopcocks (Cole-Parmer; Vernon Hills, IL). A custom designed acrylic light box was placed over the plate holder and the glass tubes were end-lighted to highlight bubble position. Bubble displacement was monitored using a Zeiss AxioCam MRc5 camera equipped with a c-mount Toyo Optics TV zoom lens (12.5 – 75 mm F1.8) and a Heliopan S 49 NL2 close-up 49 mm lens filter. Zeiss

AxioVision software (Carl Zeiss Microscopy; Jena, Germany) was utilized to control time-lapse image acquisition. Adjustments in camera height allowed the system to be calibrated to 0.1 mm/pixel.

Cell culture

Primary human endoneurial endothelial cells (pHEndECs) and simian virus (SV) 40 large T-antigen immortalized human endoneurial endothelial cells (THEndECs) were used for the *in vitro* BNB, as previously described (Yosef and Ubogu, 2013; Yosef et al., 2010). Cell culture medium for these cells consisted of RPMI-1640 medium supplemented with penicillin-streptomycin (100 IU and 100 µg/mL respectively; Corning Mediatech; Herndon, VA); 1% vitamin solution, 1% non-essential amino acid solution, 2 mM L-glutamine, 1 mM sodium pyruvate, and 10 mM HEPES buffer (all from Life Technologies; Grand Island, NY); 10% fetal bovine serum (FBS; Atlanta Biologicals, Inc.; Flowery Branch, GA); 10% NuSerum, 50 µg/mL endothelial cell growth supplements (both from Corning Discovery Labware Inc.; Bedford, MA); 1 ng/mL recombinant human basic fibroblast growth factor (Roche Applied Sciences; Mannheim, Germany); and 10 U/mL heparin (Sigma-Aldrich; St. Louis, MO). SV40 large T-antigen immortalized human brain microvascular endothelial cells (THBMECs), a well-described BBB cell line, were obtained from Dr. Monique Stins (Stins et al., 2001) and cultured to develop an *in vitro* BBB for comparison. THBMEC culture medium consisted of RPMI-1640 medium supplemented with penicillin-streptomycin (100 IU and 100 µg/mL respectively), 1% non-essential amino acid solution, 2 mM L-glutamine, 1 mM sodium pyruvate, 10 mM HEPES buffer, 10% FBS, and 10% NuSerum. Normal, human primary adult dermal fibroblasts (paDFs) and media components were purchased from American Type Culture Collection (Manassas, VA). Fibroblasts were cultured as directed by the supplier in Fibroblast Basal Medium supplemented with 2% FBS and components from the Fibroblast Low serum Growth kit.

All cells were initially expanded on rat tail collagen-coated CellBIND[®] tissue culture plates (Corning Discovery Labware Inc.; Bedford, MA and Costar; Corning, NY) as previously described (Yosef et al., 2010). Snapwell inserts were prepared by washing with 0.02N acetic acid for 30 min and coating with 6.9 µg/cm² Type 1 rat tail collagen in the presence of 0.1% glutaraldehyde in phosphate buffered saline (PBS, pH 7.4) for 1.5 hrs. After coating, insert membranes were gently washed with PBS, incubated for 5 min with 2% glycine in PBS to neutralize any remaining glutaraldehyde, and washed twice more with PBS. Seeding density in Snapwell inserts was 2.5×10^5 cells per cm² for pHEndECs, THEndECs, and THBMECs; and 1×10^5 cells per cm² for paDFs. Cell culture medium volumes for the Snapwell, 6-well plates were adjusted to a total volume of 0.45 mL for the upper chamber and 2.6 mL for the lower chamber. To generate confluent layers, cells were grown in a humidified incubator containing 95% air and 5% CO₂ for 7 days and cell culture medium was changed every 2 days. To test the effect of a mitogen previously shown to influence BNB function *in vitro* on hydraulic conductivity, 1 ng/mL recombinant human glial derived neurotrophic factor (GDNF; Peprotech Inc.; Rocky Hill, NJ) was included in pHEndEC growth medium and added to cultured cells on Snapwell inserts with medium changed every 2 days until confluence was achieved.

Hydraulic Conductivity Measurements

On the day of the assay, the bubble tracker apparatus was assembled and the Ussing diffusion chambers pre-warmed to 37°C. Cell culture medium was aspirated from the transwell inserts with confluent cultured cells and replaced with assay buffer containing RPMI-1640 medium supplemented with 5% FBS, 20 mM HEPES, and penicillin-streptomycin (200 IU and 200 µg/mL respectively). Transwell inserts with confluent cultured cells were loaded into the EasyMount diffusion chambers and placed into the pre-heated, 6-Channel EasyMount Stand. The apparatus was filled with assay buffer, the system was tested for leaks, and a 10 µL bubble was loaded into each glass tube. A ruler was placed on the plate holder alongside the 6 glass tubes and an image was taken to confirm system calibration. Baseline measurements were taken with the media reservoir elevated 2 cm above the top of the Snapwell transwell inserts and time-lapse images of bubble displacement taken every 60 seconds for 20 minutes. This baseline height was chosen based on estimated endoneurial hydrostatic pressures. Hydraulic conductivity measurements were obtained with the media reservoir elevated 12 cm above the top of the Snapwell transwell inserts, generating a 10 cm H₂O increase in hydrostatic pressure, with images of bubble displacement similarly obtained every 60 seconds for 120 minutes. The dynamic range of bubble displacement was initially ascertained using transwell inserts with membranes plugged with glue and porous inserts lacking coating or cells. All experiments were performed on day 7 following initial cell culture.

Movement of water across confluent cell layers from the luminal chamber into the abluminal chamber resulted in a direct bubble displacement in the glass tube (Figures 1a–d). Figure 1e represents a schematic diagram of the modified EasyMount Ussing diffusion chamber. The Snapwell cell culture inserts were secured in place with an O-ring to prevent leaks and completely separate the luminal and abluminal chambers. Bubble displacement was recorded by the digital Zeiss AxioCam camera using time-lapse photography (Figure 1f). A displacement measurement at each 60 second interval was generated for each bubble tracked using the Nikon Elements tracking software. Calculations for water flux (J_v) and hydraulic conductivity (L_p) were performed as described below.

Bubble displacement within the glass tube is equivalent to the height (h) of the column of water that enters the glass tube. Since the interior diameter (d) of the precision glass tube is known (1.93 mm or 0.193 cm), the volume (V) of water displaced can be determined by using the formula:

$$V = h\pi d^2 / 4$$

Next, fluid flux (J_v) across the cellular layers for each 60 second epoch was calculated by dividing the volume displaced per interval by unit time in seconds (V/s) and the known surface area of the Snapwell tissue culture insert (1.12 cm²):

$$J_v = V/s / \text{cm}^2$$

To determine the effect of luminal hydrostatic pressure on transendothelial water flux and minimize the effect of experimental noise generated by the apparatus, the mean baseline fluid flux at 2 cm H₂O (J_{v1}) was subtracted from that at 12 cm H₂O (J_{v2}). Finally, the hydraulic conductivity (L_p) was calculated following application of a change in hydrostatic pressure (ΔP) of 10 cm H₂O as follows:

$$L_p = (J_{v2} - J_{v1}) / \Delta P$$

Data Acquisition and Analysis

Data acquisition was performed using the NIS-Elements Advanced Research software with Tracking Module (Nikon Instruments Inc.; Melville, NY). Briefly, background was subtracted using the last image recorded as the reference image. Each bubble was marked as a region of interest and tracked throughout the length of the experiment. Bubble displacement was measured every 60 seconds. Fluid (water) flux and hydraulic conductivity were deduced as described above. Statistical analyses were performed and graphs were generated using Prism software (GraphPad Software, Inc.; La Jolla, CA). Unless otherwise stated, all data are expressed as mean $\pm$ standard error of mean (SEM). Significance was determined using the unpaired Welch's t-test, with $p < 0.05$ indicative of statistical significance.

RESULTS

BNB hydraulic conductivity

The mean hydraulic conductivity was determined to be $2.00 \pm 0.19 \times 10^{-7}$ cm/s/cm H₂O for pHEndECs, $2.17 \pm 0.44 \times 10^{-7}$ cm/s/cm H₂O for THEndECs, $0.20 \pm 0.10 \times 10^{-7}$ cm/s/cm H₂O for THBMECs and $3.77 \pm 0.12 \times 10^{-7}$ cm/s/cm H₂O for paDF (Figure 2). There was no significant difference in hydraulic conductivity comparing the BNB using primary and immortalized cells, implying retention of this essential biophysical property with cellular immortalization. The BNB had a mean 1.9-fold higher resistance than the water impervious paDFs but 10-fold less resistance than the BBB to hydrostatic pressure-induced water flux *in vitro* based on these measurements.

Role of GDNF in regulating BNB hydraulic conductivity

We have previously found that GDNF treatment of pHEndECs promoted an increase in transendothelial electrical resistance and a decrease in permeability to high molecular weight dextran 48 hours following serum withdrawal from confluent cultures that induced diffuse endothelial cell injury and cellular detachment (Yosef and Ubogu, 2012). To determine whether GDNF-mediated enhancement of endothelial barrier function may affect hydraulic conductivity during initial barrier formation, GDNF was added at 1 ng/ml to pHEndECs in addition to regular culture medium. Under these conditions, the hydraulic conductivity was slightly lower for GDNF-treated BNB ($1.71 \pm 0.27 \times 10^{-7}$ cm/s/cm H₂O) compared to the regular growth medium cultured BNB ($2.00 \pm 0.19 \times 10^{-7}$), representing a 14.5% mean reduction.

DISCUSSION

We developed an experimental system to reproducibly measure water flux across endothelial cell layers using predominantly commercially available components. The system was designed such that raising the reservoir would apply a hydrostatic pressure gradient across the Snapwell insert membranes containing the endothelial cell layer from the luminal to the abluminal side. The mean BNB hydraulic conductivity is comparable to measurements previously published for restrictive microvascular endothelial cell lines, and significantly lower than macrovascular endothelial cell lines *in vitro* (Chang et al., 2000; Dull et al., 2001; Hippenstiel et al., 1997; Hubert et al., 2006; Li et al., 2010; McCandless et al., 1991; Qiao et al., 1993; Yaccino et al., 1997), as shown in Table 1. We utilized paDFs that form water impervious barriers but do not form tight junctions to aid with our comparative analyses. Compared to several previously published devices (Lopez-Quintero et al., 2009; Mathura et al., 2014), our system applied positive hydrostatic pressure gradients on the luminal side of endothelial cell layers, mimicking changes in microvascular pressure, rather than negative pressure applied on the abluminal side that mimics a reduction in interstitial pressure. In addition, we used standardized time-lapse digital photography to detect and quantify bubble displacement (Hubert et al., 2006) using commercially available software rather than a motorized bubble tracker device.

As may be expected, the mean hydraulic conductivity for THBMECs that form the BBB was the lowest *in vitro* value measured or published to date. Comparing the *in vitro* human BBB and BNB, there was a 10-fold reduction in hydraulic conductivity at the BBB, implying a much higher resistance to transendothelial water flux in the brain compared to peripheral nerves. These observations are consistent with the BBB being the most restrictive mammalian microvascular barrier, with the BNB serving as the second most restrictive (Allt and Lawrenson, 2000; Poduslo et al., 1994; Ubogu, 2013; Yosef and Ubogu, 2013). The low BBB hydraulic conductivity may serve to critically maintain ionic concentrations required for complex signaling needed for cerebral function. Furthermore, this biophysical property may also serve to resist the development of cerebral edema following changes in intracranial vascular pressure, as brain volume expansion is restricted by its location within the rigid skull.

The hydraulic conductivity system provides an avenue for comparative studies utilizing mitogens and small molecule inhibitors to determine the determinants and signaling pathways responsible for transendothelial water flux at the BNB and BBB. This will provide important knowledge of an essential restrictive microvascular biophysical property, in addition to transendothelial electrical resistance and solute permeability to small and large molecules (Ubogu, 2013; Yosef and Ubogu, 2012; Yosef and Ubogu, 2013; Yosef et al., 2010). Further studies are required to determine the effect of GDNF and other mitogens such as basic fibroblast growth factor, transforming growth factor- β , vascular endothelial growth factor and hydrocortisone on BNB water flux. It is currently unknown whether hydraulic conductivity at the BNB or BBB is facilitated by specific water channels (such as aquaporin 1) (Nguyen et al., 2015) providing a transcellular route of entry or via transcellular or paracellular osmosis. In future studies, gene knock-down could be employed to manipulate tight junction, transporter, and ionic/water channel protein levels using pHEndECs or

THEndECs, or THBMECs to gain insights to their roles in BNB and BBB hydraulic conductivity respectively. It is also unknown whether transendothelial water efflux (i.e. from the abluminal to luminal side) may occur across the BBB and BNB *in vitro*.

Culturing uniformly confluent endothelial monolayers on transwell insert membranes was the primary obstacle observed with the hydraulic conductivity model, as our endothelial cells grow more uniformly on CellBIND® tissue culture plates. Our system design allows up to 6 simultaneous and independent bubble displacement measurements from individual Snapwell inserts, facilitating more accurate hydraulic conductivity measures of a single experimental condition, as well as direct comparison of several experimental conditions applied to the endothelial cells prior to Snapwell insert attachment to the diffusion chamber at the same time. Time-lapse digital photography with the use of sensitive particle tracking software aided with data accuracy, supported by the small SEM values. The cell layers remained viable throughout the duration of the experiment, aided by the circulating water bath maintaining internal temperatures at 37°C, as evidenced by uniform bubble displacements.

In conclusion, we developed a hydraulic conductivity system and determined values for the human BNB and BBB *in vitro*. While we utilized some custom manufactured or adapted components that are described in detail, the commercially available EasyMount 6-Chamber diffusion chamber is a standardized component that should allow biomedical laboratories pursue these studies. Our adaptations provide a method of modifying the diffusion system to measure unidirectional fluid flux that mimics transendothelial water movement from microvessel lumen into tissue parenchyma. The particle tracking software is also commercially available and is easily applied to standardized time-lapse digital photography. Further studies are planned to further characterize the human BNB and BBB *in vitro* using this model system.

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Non-standard abbreviations

paDF	primary adult dermal fibroblasts
pHEndECs	primary human endoneurial endothelial cells
THBMECs	SV40 large T-antigen immortalized human brain microvascular endothelial cells
THEndECs	SV40 large T-antigen immortalized human endoneurial endothelial cells

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HIGHLIGHTS

Experimental apparatus and methods to measure hydraulic conductivity are described.

An adaptable diffusion chamber and digital camera-based bubble tracker were used.

The human blood-nerve and blood-brain barrier hydraulic conductivities were obtained.

Cellular immortalization does not affect blood-nerve barrier water flux *in vitro*.

Reliable mechanistic studies of blood-nerve barrier water flux can be performed.

Figure 1. Hydraulic conductivity measurement apparatus

Photographs of the hydraulic conductivity system, primarily consisting of a diffusion chamber connected to hydrostatic pressure reservoir, circulating water bath and bubble tracker apparatus utilizing digital time-lapse photography are shown. Figure 1a shows the experimental set-up without tubing connecting the hydrostatic reservoirs to the diffusion chambers. Figure 1b shows the set-up with the water bath disconnected and tubing from the hydrostatic pressure reservoir attached, while Figure 1c has the light box removed to clearly demonstrate the slotted plate containing precision bore glass tubes used to detect bubble displacement. Figure 1d is a flow diagram depicting the movement of medium through the hydraulic conductivity system with Figure 1e illustrating the modified EasyMount Ussing diffusion chamber with sealed fill ports (X). Black arrows depict the movement of medium into the chamber from the hydrostatic pressure reservoir and out to the glass tubing of the bubble tracker device, while white arrows indicate water flux across the Snapwell insert membranes with cultured confluent cells. Figure 1f is a schematic representation of the assembled bubble tracker device with bubbles (white circles) inserted into glass tubes and camera suspended overhead to record bubble displacement with a light box to illuminate the slotted plate and eliminate external light. Black arrows indicate fluid movement. Key: (1) adjustable height, 6-channel, hydrostatic pressure reservoir with 10 cc syringes, (2) aluminum breadboard, (3) 6-channel EasyMount stand, (4) EasyMount Ussing diffusion chamber with Snapwell inserts, (5) circulating, heated water bath, (6) slotted plate for holding 6 precision bore glass tubes, (7) light box, (8) camera mount, and (9) digital camera.

Figure 2. Hydraulic conductivity of the human blood-nerve and blood-brain barriers *in vitro*
 Hydraulic conductivity experiments were performed on day 7 of culture as described in the Materials and Methods section in triplicate. Statistically significant differences in hydraulic conductivity (L_p) are observed between the paDF, pHEndEC, THEndEC, THBMEC layers, with no difference between the pHEndEC and THEndEC layers. THBMEC forms the most resistant barrier to transendothelial water flux while immortalization does not significantly

affect hydraulic conductivities of BNB-forming cell lines *in vitro*. * indicates $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and n.s. indicates not significant.

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Table 1
Comparison of BNB hydraulic conductivity with other mammalian cell lines *in vitro*

Data obtained from the human BNB and BBB cell lines are compared with published data from other mammalian cell lines. The table demonstrates the highly restrictive nature of confluent neural microvascular endothelial cells (THEndEC, pHEndEC and THBMEC) to transendothelial water flux, depicted as low hydraulic conductivity values, with the THBMEC that form the BBB being the most restrictive.

CELL TYPE (and abbreviated designation)		Hydraulic Conductivity (L_p) ($\times 10^{-7}$ cm/s/cm H_2O) (mean $\pm$ SE)	References
OPAEC	Ovine pulmonary artery endothelial cells	250 $\pm$ 110	McCandless et al. 1991
BPMVEC	Bovine pulmonary microvessel endothelial cells	26.0 $\pm$ 5.0	Qiao et al. 1995
PAEC	Porcine pulmonary artery endothelial cells	20.0 $\pm$ 10	Hippenstiel et al. 1997
ACS	primary rat astrocytes	5.0 $\pm$ 2.7	Li et al. 2010
paDF	Primary adult dermal fibroblasts	3.77 $\pm$ 0.12	Reported here
BAEC	Bovine aortic endothelial cells	3.58 $\pm$ 0.18 to 3.60 $\pm$ 0.20	Chang et al. 2000, Dull et al. 2001
HUVEC	Human umbilical vein endothelial cells	2.85 $\pm$ 0.30 to 3.55 $\pm$ 0.39	Chang et al. 2000, Dull et al. 2001
RMEC	Retinal microvascular endothelial cells (bovine)	1.44 $\pm$ 0.26 to 7.82 $\pm$ 0.85	Yaccino et al. 1997
BREC	Bovine retinal microvascular cells	2.79 $\pm$ 0.33	Chang et al. 2000
BLMVEC	Bovine lung microvascular endothelial cells	2.40 $\pm$ 0.50	Hubert et al. 2006
THEndEC	Immortalized human endoneurial endothelial cells	2.17 $\pm$ 0.44	Reported here
pHEndEC	Primary human endoneurial endothelial cells	2.00 $\pm$ 0.19	Reported here
bEnd.3	immortalized mouse brain microvascular endothelial cell line	1.7 $\pm$ 1.1	Li et al. 2010
bEnd.3 and ACS	bEnd.3 and astrocyte co-culture	1.4 $\pm$ 0.6	Li et al. 2010
THBMEC	Immortalized human brain microvascular endothelial cells	0.20 $\pm$ 0.10	Reported here