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Analysis of Endothelial Barrier Function In Vitro

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Abstract

Increased microvascular solute permeability underlies many forms of pathophysiological conditions, including inflammation. Endothelial monolayer cultures provide an excellent model system which allows systemic and mechanistic study of endothelial barrier function and paracellular permeability in vitro. The endothelial-specific *complexus adherens* junction protein VE-cadherin and their intracellular complex form pericellular structures along the cell borders which are critical to regulate endothelial barrier function by controlling pericellular permeability of vasculature. Here, we describe methods for both visualizing and quantifying junctional permeability and barrier changes in endothelial monolayers in vitro.

Keywords

Endothelial permeability; Barrier function; VE-cadherin; TEER; Microcarrier beads; Cell-column chromatography

1. Introduction

The vascular endothelium is a semipermeable barrier, which creates and separates the tissue and vascular compartments. Endothelial monolayers regulate the exchange of fluid and solutes between the blood and the interstitial space. The integrity of vascular endothelial junctions is perhaps the most important structural feature controlling bulk exchange; therefore, in vitro endothelial cultures provide a simple and powerful model of in vivo microvascular barrier that has been demonstrated to represent many of the inflammatory responses associated with clinical disease states. Increased microvascular solute permeability underlies the pathophysiology of many acute and chronic forms of inflammation, wound healing, and immune responses. In this chapter, we describe several well-characterized in vitro techniques commonly used to study endothelial permeability/barrier function.

2. Materials

2.1. Immunofluorescent Staining for VE-Cadherin

1. Vascular endothelial cells from different anatomic and species origins may be used in these experiments.
2. Medium can be either endothelial cell growth medium (EGM, Lonza Walkersville, Inc. Walkersville, MD), Medium199 supplemented with L-

glutamine, 20% fetal bovine serum (FBS), 1% penicillin, streptomycin and amphotericin (Sigma, St. Louis, MO), or appropriate medium for the cell type being studied.

3. Dulbecco's Phosphate Buffered Saline (DPBS) and Hank's Balanced Salt Solution (HBSS) (Sigma).
4. Fibronectin is dissolved in HBSS at 25 µg/ml and is used to coat all cell-culture surfaces. Fibronectin is supplied by Biomedical Technologies Inc. (BTI, Boston, MA) at a stock concentration of 1 mg/vial.
5. Staining/incubation/wash buffer (0.1% evaporated milk in DPBS).
6. Full strength trypsin-EDTA from Cellgro, Mediatech Inc. (Manassas, VA) – typically diluted 1:5 with PBS for final working solution.
7. Glass coverslips (12 mm diameter and 1.0 mm thickness) (Fisher, Rochester, NY).
8. Corning Costar 24-well cluster cell culture plates, Petri dish (100 mm) (Fisher).
9. 95% ethanol (Fisher).
10. Primary antibody: monoclonal mouse anti-human VE-cadherin antibody (Immunotech, Westbrook, ME).
11. Cy3 donkey anti-mouse immunoglobulin (IgG) (H + L) fluorescent-labeled antibody (Jackson ImmunoResearch Laboratories Inc. Westgrove, PA).
12. Coverslip forceps/tweezers.
13. Fluorescent/confocal microscope.

2.2. Endothelial Electric Resistance Measurement

1. See Subheading 2.1, items 1, 2, 4, and 8 for endothelial cells, cell growth medium, fibronectin, and 24-well cluster cell culture plates.
2. BD Falcon polycarbonate transwell inserts (8 µm pore size for 24 well/plate) are from VWR International (West Chester, PA).
3. Endohm EVOM endothelial ohmmeter (World Precision Instrument, Sarasota, FL).

2.3. Horseradish Permeability Assay

1. See Subheading 2.1, items 1, 2, 4, and 8 for endothelial cells, cell growth medium, fibronectin, and 24-well cluster cell culture plates.
2. See subheading 2.2 for polycarbonate transwell insert.

3. Horseradish peroxidase (HRP, VI-A type, 44,000 MW, Sigma): standard curve for HRP is prepared in a range of 0–50 μ M in distilled/deionized H₂O.
4. Sodium phosphate buffer (NaH₂PO₄ 50 mM), guaiacol, and hydrogen peroxide (H₂O₂) (Sigma).
5. Glass tubes (Borosilicate disposable culture tube, 12 $\times$ 75 mm, Fisher).
6. Semimicro disposable plastic cuvettes (10 mm light path, VWR International).
7. Spectrophotometer.

2.4. Cell Column Chromatography

1. See Subheading 2.1, items 1 and 2 for endothelial cells and cell growth medium.
2. Cytodex-3 microcarrier beads (Sigma) in Ca²⁺/Mg²⁺ free PBS.
3. 0.5 L Bellco stir flasks and flask stirring device (Vineland, NJ).
4. 30 min graduations on: off 120VAC timer.
5. Column-injectate tracer mixture: 2% blue dextran (MW 2,000 kDa), 0.2% cyanocobalamin (Vit B12, MW 1.355 kDa), and 0.0125% sodium fluorescein (MW 0.376 kDa) in HBSS (see ^{Note 1}).
6. Gilson minipuls peristaltic pump (0.9–3 ml/min) (Gilson, Middleton, WI).
7. Omni water jacked 6.6 mm ID glass column (Omni, Minneapolis, MN) fitted with a 70 μ m frit to restrict beads.
8. 50 μ l in-line injector port (Rainin, Oakland, CA).
9. Gilson fraction collector (Gilson).
10. Flat-bottom 96-well plates (Nunc, Rochester, NY).

2.5. Trypan Blue Albumin Permeability Analysis

1. See Subheading 2.1, items 1 and 2 for endothelial cells, cell growth medium.
2. See Subheading 2.4, items 2–4 for Cytodex-3 microcarrier bead, Bellco stirring flasks, and on:off control timer.
3. Trypan blue (Sigma), bovine serum albumin (BSA), Cohn Frac V (fatty acid free) (Sigma).
4. Dibutyl phthalate and dioctyl phthalate (1:1 mixture) (Sigma) (see ^{Note 2}).
5. Narrow format stiletto plastic tubes (1 ml) (Fisher).

¹Do not filter sterilize column injectate.

²Caution: Dibutyl: dioctyl phthalate is toxic! Avoid skin contact!

6. Microcentrifuge (Fisher).
7. 96-well plate reader.

3. Methods

3.1. Immunofluorescent Staining for VE-Cadherin

VE-cadherin is an endothelial *complexus adherens*-specific junction adhesion molecule located at interendothelial cell junctions. VE-cadherin plays a fundamental role in maintaining endothelial integrity, barrier function, and leukocyte extravasation. VE-cadherin also regulates cell proliferation, apoptosis, and modulates VEGF-receptor signaling. Loss of VE-cadherin expression from/or disorganized VE-cadherin distribution at cell junctions directly influences monolayer integrity and endothelial permeability. Consequently, immunostaining for VE-cadherin in endothelial cells is used intensively in endothelial biology to provide structural correlates to barrier changes. The method described below can be used to visualize VE-cadherin distribution in endothelial cells grown on glass coverslips in 24-well/plates in response to barrier modulators.

1. When endothelial cultures are near confluency, passage cells into 24-well/plates with glass coverslips preloaded in the plates (1).
2. To sterilize glass coverslips, put coverslips into a Petri dish with 95% ethanol (30 min). Transfer coverslips to a new sterile Petri dish and air dry coverslips under UV light in a biosafety cabinet; place one coverslip into each well of the 24-well/plate.
3. Coat coverslips with 100 μ l of fibronectin at 25 μ g/ml for 5 min (see Note ³).
4. Trypsinize ECs with Trypsin-EDTA, and resuspend cells in desired amount of endothelial growth medium. Seed the cells (1×10^5 cells/well) onto fibronectin-coated coverslips.
5. Place the plate in a 37°C incubator with 100% humidity and 5% CO₂/air. Replace medium every other day. It usually takes 3–4 days for cells to reach confluency.
6. On the day of experiment, treat cells with appropriate stimuli for experiments.
7. Fix cells with 1 ml of cold (–20°C) 95% ethanol for 30 min in 4°C refrigerator (see Note ⁴). Discard ethanol and add 0.5 ml of 100% acetone to permeabilize cells for 1 min, discard acetone, and air dry coverslips for 3 min.
8. Incubate coverslips with 250 μ l of primary anti-VE-cadherin antibody with a concentration of 1 μ g/ml in DPBS with 0.1% milk for 2 h at RT.

³Fibronectin can be recovered and reused at least twice.

⁴Fixed cells/coverslips can be stored in 95% ethanol at 4°C overnight and stained next day.

9. Discard primary antibody solution and wash coverslips 3× with DPBS containing 0.1% milk.
10. Incubate coverslips with 250 µl of Cy3 donkey anti-mouse immunoglobulin G (H + L) (secondary antibody) at a dilution of ~ 1:200 for 2 h at RT.
11. Discard secondary antibody solution and wash coverslips 3× with DPBS containing 0.1% milk.
12. Move coverslips from the plate using coverslip forceps. Dry coverslips with cell side face up and mount coverslips with Vectashield mounting medium with cell side down on glass slide (see ^{Note 5}).
13. View stained cells by fluorescent microscopy. An example of VE-cadherin staining is shown in Fig. 1. The distribution of VE-cadherin is a continuous and zigzag pattern localized at the cell-contact region (cell junction).

3.2. Trans-Endothelial Electrical Resistance Measurements

Measurement of endothelial electrical resistance (ER) is a simple and direct method to study endothelial barrier function in vitro. The basic concept is that when cells grow to confluency and become tightly connected, they resist the passage of electrical current, and a property that can be easily measured using an Endohm EVOM endothelial ohmmeter. Trans-endothelial electrical resistance (TEER) measurement provides a quick and realtime evaluation of the integrity of endothelial monolayers. The method described below is intended specifically for measuring TEERs with endothelial cells grown in a 24-well cell inserts.

1. Place polycarbonate inserts in 24-well cluster plate and coat inserts with 0.2 ml of fibronectin (25 mg/ml) per insert.
2. Trypsinize ECs with Trypsin-EDTA, resuspend cells with endothelial growth medium with density of 1×10^5 cells/ml; seed 0.5 ml of cell suspension (5×10^4 cells/per insert) into an insert (upper chamber), and add 1.0 ml of medium per well (lower chamber).
3. Place the plate in a 37°C incubator with 100% humidity and 5% CO₂/air. Change medium the next day. Cells usually reach confluency in 3 days. Examine cells under microscope to confirm cell confluency by phase contrast.
4. On the day of experiment, remove medium from both upper and lower chambers, add 0.8 ml of prewarmed (37°C) fresh medium to the upper chamber and 1.0 ml of medium to the lower chamber (see ^{Note 6}).

⁵Use mounting medium with DAPI counterstain to visualize nuclear DNA in cells.

⁶We usually perform endothelial electrical resistance measurements and HRP permeability assay simultaneously.

5. Place the plate in a 37°C incubator for 1 h and let the cells to equilibrate and then measure the baseline electrical resistance with Endohm EVOM endothelial ohmmeter. In general, confluent endothelial cells grown in 8 μm pore size have a resistance reading between 400 and 420 Ω (see Note 7).
6. Add stimuli to the upper chamber. Measure electrical resistance (in triplicate) per well and record values at 1, 2, and 3 h, etc. The mean value is then used for final data calculation.
7. For TEER calculations, the values obtained from a blank insert (no cells) is subtracted to give the net resistance. This is then multiplied by the insert membrane area to give the resistance in area-corrected units and is expressed as $\Omega \cdot \text{cm}^2$, taking into account the surface area of the filter (see Note 8).

3.3. Horseradish Peroxidase Permeability Assay

There are a number of reagents that have been described as markers/tracers used for evaluating endothelial permeability, such as I^{125} -labeled BSA, fluorescein-isothiocyanate (FITC)-conjugated dextran, ^3H -mannitol, ^{14}C -labeled dextran, and ^{14}C -inulin, etc. Here, we describe a simple and sensitive method using HRP as a tracer to measure endothelial permeability. HRP activity can be inhibited by sodium azide (2). The enzymatic reaction can be initiated by adding hydrogen peroxide (H_2O_2) and a substrate (e.g., guaiacol) in the testing samples. The changes of HRP activity in the samples can then be easily monitored by a UV spectrophotometer in vitro (2, 3) (see Note 9). The assay is carried out using 8.0 μm pore size polycarbonate cell culture inserts that fit in 24-well cluster cell culture plate (see Note 10). All reagents should be made fresh prior to the assay.

1. See steps 1–3 of Subheading 3.2 above for the preparation of fibronectin-coated inserts, passing cells, and culture methods. Endothelial electric resistance will help to determine the level of confluence (see Note 6).
2. On the day of the experiment, remove medium from both upper and lower chambers. Add 0.8 ml of prewarmed (37°C) medium containing HRP (0.126 μM) and permeability treatments to the upper chambers and 1.0 ml of medium in the lower chambers.
3. Place the plate in an incubator and at 1, 2, 3, and 6 h intervals, collect a 50 μl aliquot of medium from the lower chamber. Store samples in borosilicate disposable tubes, and keep on ice until assaying.

⁷We do not recommend performing experiments with an initial baseline resistance reading less than 400 Ω .

⁸Check the insert membrane area before calculation. The insert membrane area could be different if inserts are from different suppliers.

⁹The advantages of using HRP over other markers/tracers (I^{125} -labeled BSA, ^{14}C -labeled dextran, ^{14}C -inulin, and FITC-conjugate dextran) are that no radio-labeled materials are involved in the assay and a visible range spectrophotometer is more common than fluorescent spectrometers.

¹⁰We recommend using transparent inserts that allow visualization of live cells by light microscopy. Inserts with 3 or 0.4 μm pore size can also be used based on experimental needs.

4. Prepare a standard curve in the range of 0–50 μM in distilled/deionized H_2O .
5. To measure HRP activity, add 25 μl of collected samples or standard to 860 μl of reaction buffer containing 50 mM NaHPO_4 and 5 mM guaiacol in 2 ml glass tubes. Vortex samples. HRP standard curve should be 0, 0.5, 1.0, 2.5, 5.0, 10, and 25 μM . All samples should be assayed in duplicate.
6. Start the reaction by addition of 100 μl hydrogen peroxide reaction solution (0.6 mM in distilled/deionized H_2O) to the sample and standard tubes and incubate the tubes at RT for 30 min (see ^{Note 11}).
7. After reaction, transfer samples to semimicro disposable cuvettes and read the sample absorbance at 470 nm by spectrophotometer, and record the OD number for data calculation.
8. Data are calculated as follows: $\text{OD}_{470} = \text{sample OD}_{470} - \text{blank OD}_{470}$. Data are expressed as OD_{470} for permeation of HRP across transwell filters. Figure 2 shows endothelial permeability data of cells treated with platelet activating factor (PAF) simultaneously measured by both TEER and HRP permeability assays [4].

3.4. Cell-Column Chromatography Permeability Assay

Cell-column chromatography is a sensitive and high throughput method for evaluating endothelial monolayer permeability of cells grown on microcarriers (beads) to small molecular weight solutes using tracers, including blue dextran, cyanocobalamin, and sodium fluorescein (5) (see ^{Note 12}). The advantages of microcarrier cultures include exposure of cultured cells to continuous fluid shear, which may more closely mimic an in vivo environment. Secondly, defects of endothelial monolayers on individual microcarriers can be negligible on the total response. Importantly, the duration of each round of permeability analysis can be completed in ~3 min. Therefore, multiple tests can be performed simultaneously within the course of the experiment. Finally, repetitive testing on single batches of cells allows for powerful statistical approaches, e.g., repeated measures analysis of variance to be used. Endothelial cells derived from different organs have been cultured to confluence on microcarrier beads to study endothelial barrier function/permeability using this cell-column chromatography permeability assay (5–9).

1. Cytodex-3 microcarriers are cultured with endothelial cells by mixing 3 ml of gelatin-covered microcarrier beads with 50 ml of cell-culture medium. Cells, beads, and medium are placed in a Bellco™ stir flask at an

¹¹ H_2O_2 reaction solution must be prepared just before the assay.

¹²To determine permeability in this model (5), it is assumed that elution profiles of permeant tracers (sodium fluorescein, cyanocobalamin) depend on the properties of the moving, fluid phase of the column, the endothelial junctional (pericellular) permeability properties of the endothelial monolayer and the diffusion characteristics of these permeant tracers within the microcarrier beads' matrix. In contrast to the permeant tracers, the elution of blue dextran reflects only on the movement of the fluid phase through the column. A modified Marquardt iteration scheme is used to estimate monolayer permeability that best fits the experimental data. This "best fit" is determined by the minimization of the coefficient of variation between a computer-generated prediction of the permeant tracer's elution profile and the experimentally observed elution profile.

- endothelial cell density of 1:4 (assuming that each ml of Cytodex-3 beads has a total surface area of 328 cm²).
2. Cultures are stirred at 60 rpm intermittently (30:30 min, on:off) for 24 h. Medium is then adjusted to 100 ml, and then stirred continuously at 60 rpm.
 3. Replace 50% of culture medium every 3 days. Cell confluence is examined visually by phase-microscopic inspection of microcarrier beads.
 4. On the day of assay, cell-covered beads are transferred into a column volume of 0.67 cm³, which provides ~130 cm² of endothelial cell culture surface (~6.5 × 10⁶ cells).
 5. Cells are washed and equilibrated with HBSS containing 0.5% BSA and 10 mM HEPES (pH 7.4). Constant pressure perfusion is maintained by a Gilson peristaltic pump at 0.9 ml/min (this rate was chosen to match the spontaneous column flow rate induced by gravity).
 6. At time points, permeability measurements are made by injecting a 50 µl bolus of mixed tracers (blue dextran/sodium fluorescein/cyanocobalamin, see Subheading 2.4 item 5) by an in-line rotary 50 µl injection loop. The cell column and all perfusate solutions are maintained at 37°C.
 7. A Gilson fraction collector (model 203) equipped with a drop counter is used to collect elutes into 96-well/plate: two drops of elutes per well in the initial 24 wells and then six drops per well for the next 22 wells (see ^{Note 13}). A total of 46 elutes is collected per cell column. This approach not only provides higher resolution of tracers in the early (steep) portion of elution curves, but also collects enough samples to recover ~100% of all applied tracers.
 8. Columns can then be washed out with HBSS containing 0.5% BSA and 10 mM HEPES (pH 7.4) for at least 5 min.
 9. Repeat steps 6–8 for replicate testing of each column (see ^{Note 14}).
 10. Read the plate at 620 nm for blue dextran, 540 nm for cyanocobalamin, and 492 nm for sodium fluorescein on a spectrophotometric plate reader and store the data on a computer for later analysis. Figure 3 shows an example of typical profiles of blue dextran, cyanocobalamin, and sodium fluorescein elution profiles under normal (a) and increased permeability (b) (reduced barrier) conditions and scanning microscopy of endothelial cells grown on microbeads (c and d).

¹³The order of tracer elutes is based on the molecular weight of the tracers: (1) blue dextran with molecular weight of 2,000 kDa cannot penetrate the bead matrix. Therefore, it follows the fluid phase and is considered flow tracer; (2) sodium fluorescein with molecular weight of 0.376 kDa and (3) cyanocobalamin with molecular weight of 1.355 kDa can permeate the cell layer and diffuse into the bead matrix beneath the cells and exhibit delayed exit from the column if increased endothelial permeability occurs which is proportionate to monolayer barrier properties.

¹⁴We recommend testing each sample in triplicate.

3.5. Trypan-Blue Dye Extraction Permeability Assay

The extraction of trypan blue-albumin complexes by cell-covered microcarriers is another simple method for the measurement of in vitro barrier function. The original approach (10) has been modified by several laboratories (11, 12). It is estimated a stoichiometric maximal binding of 37 trypan blue molecules per albumin, and that >95% of trypan blue is bound to albumin as a 106 kDa complex (11). Thus, cell-covered microcarrier beads absorb trypan blue-albumin dye complexes proportionate with endothelial monolayer barrier properties.

1. Microcarrier cell cultures are accomplished as described in Subheading 3, 4 (above).
2. Bellco stir flasks containing cell-covered microcarrier are removed from the incubator and allowed to settle.
3. An aliquot of 300 μ l confluent cell-covered microcarrier beads in 2 ml of culture medium are transferred to a 3 ml plastic capped Eppendorf tube. Beads will fall to the tube bottom by gravity in 15 s.
4. Culture medium is replaced with test medium; alternatively, test agents can be added ("spiked") into medium.
5. Tubes containing microcarrier beads are then continuously and gently agitated at 37°C. An aliquot of 200 μ l of trypan blue/albumin complex (TBA: 0.4% trypan blue, 0.5% BSA in PBS) is then added to each tube, and the tubes are recapped. Trypan blue dye absorption by microcarrier beads progressively extracts TBA from the reaction mix. Maximal dye uptake is usually achieved within 60 min. If monolayer barrier function is decreased, the rate of dye uptake is increased, approaching that of naked (non-cell covered) microcarriers.
6. At time points, the tubes are rapidly opened and a 200 μ l aliquot of mixed bead/dye is removed and centrifuged on a cushion of 400 μ l of 1:1 dibutyl:dioctyl phthalate in narrow format Eppendorf tube ("stiletto" tube) effectively separating the beads from the dye in solution to end uptake of tracer dye by beads at each time point by centrifugation at $10,000 \times g$ for 30 s (see Note 14).
7. The supernatant containing TBA is then diluted tenfold, and the absorbance of the sample is read at 620 nm by a spectrophotometer.
8. Trypan blue dye uptake permeability results are expressed as the inverse of the diluted supernatant absorbance (1/Abs vs. time). Appropriate controls include non-cell covered microcarriers that provide a measurement of maximal rate of dye uptake by microcarriers.

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References

1. Wang Y, Gu Y, Granger DN, Roberts JM, Alexander JS. Endothelial junctional protein redistribution and increased monolayer permeability in HUVECs isolated during preeclampsia. *Am J Obstet Gynecol.* 2002; 186:214–220. [PubMed: 11854638]
2. Ortiz de Montellano PR, David SK, Ator MA, Tew D. Mechanism-based inactivation of horseradish peroxidase by sodium azide. Formation of *meso*-Axioporphyrin IX. *Biochemistry.* 1988; 27:5470–5476. [PubMed: 3179265]
3. Lampugnani MG, Resnati M, Raiteri M, Pigott R, Pisacane A, Houen G, Ruco LP, DeJana E. A novel endothelial-specific membrane protein is a marker of cell-cell contacts. *J Cell Biol.* 1992; 118:1511–1522. [PubMed: 1522121]
4. Zhang Y, Gu Y, Lucas MJ, Wang Y. Antioxidant superoxide dismutase attenuates increased endothelial permeability induced by platelet-activating factor. *J Soc Gynecol Investig.* 2003; 10:5–10.
5. Haselton FR, Mueller SN, Howell RE, Levine EM, Fishman AP. Chromatographic demonstration of reversible changes in endothelial permeability. *J Appl Physiol.* 1989; 67(5):2032–48. [PubMed: 2480948]
6. Alexander JS, Hechtman HB, Shepro D. Phalloidin enhances endothelial barrier function and reduces inflammatory permeability in vitro. *Microvasc Res.* 1988; 35(3):308–15. [PubMed: 3393092]
7. Alexander JS, Blaschuk OW, Haselton FR. An N-cadherin-like protein contributes to solute barrier maintenance in cultured endothelium. *J Cell Physiol.* 1993; 156(3):610–8. [PubMed: 8360263]
8. Haselton FR, Dworska E, Evans SS, Hoffman LH, Alexander JS. Modulation of retinal endothelial barrier in an in vitro model of the retinal microvasculature. *Exp Eye Res.* 1996a; 63(2):211–22. [PubMed: 8983979]
9. Haselton FR, Woodall JH, Alexander JS. Neutrophil-endothelial interactions in a cell-column model of the microvasculature: effects of fMLP. *Microcirculation.* 1996b; 3(3):329–42. [PubMed: 8930889]
10. Boiadjeva S, Hallberg C, Höglström M, Busch C. Methods in laboratory investigation. Exclusion of trypan blue from microcarriers by endothelial cells: an in vitro barrier function test. *Lab Invest.* 1984; 50(2):239–46. [PubMed: 6694362]
11. Bottaro D, Shepro D, Peterson S, Hechtman HB. Serotonin, norepinephrine, and histamine mediation of endothelial cell barrier function in vitro. *J Cell Physiol.* 1986; 128(2):189–94. [PubMed: 2942553]
12. Alexander JS, Patton WF, Christman BW, Cuiper LL, Haselton FR. Platelet-derived lysophosphatidic acid decreases endothelial permeability in vitro. *Am J Physiol.* 1998; 274(1 Pt 2):H115–22. [PubMed: 9458859]

Fig. 1. VE-cadherin staining in endothelial cells (ECs) grown on glass coverslips. **(a)** Control ECs; **(b)** cells treated with platelet-activating factor; and **(c)** cells after 24 h recovery. In control cells, VE-cadherin is only stained at cell borders, exhibits zigzag continuous expression pattern **(a)**. In contrast, intercellular gaps are present at cell contact regions in ECs treated with PAF **(b)**. Disorganized VE-cadherin distribution in endothelial cells is recovered after PAF was removed from the culture indicating that microenvironment condition is critical to maintain junction protein VE-cadherin expression and distribution and EC integrity.

Fig. 2.

Endothelial permeability simultaneously measured by TEER and HRP permeability assays in endothelial cells treated with platelet activating factor (PAF). Please note that reduced endothelial electrical resistance is correlated with increased HRP leakage in cells grown on cell culture inserts when cells are treated with PAF.

Fig. 3. A representative data from cell-column chromatography permeability assay. (a) and (b) show typical profiles of blue dextran, cyanocobalamin, and sodium fluorescein eluate under normal (a) and increased permeability (b) conditions. (c) and (d) show scanning microscopy of endothelial cells reach confluence grown on microcarrier (*beads*).