Technical Report

Digital imaging system and virtual instrument platform for measuring hydraulic conductivity of vascular endothelial monolayers

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Abstract

We have developed an automated, digital imaging system, controlled by two virtual instruments, to measure hydraulic conductivity (L_p) of cultured endothelial monolayers. Live digital images of multiple independent experiments were captured by custom-designed video processing software running in National Instruments LabVIEW 6.1. Fluid displacement data are automatically displayed in real time as both volumetric flux (J_v) and hydraulic conductivity (L_p). A separate data analysis program is used to display permeability values from stored displacement measurements and displays J_v or L_p of each monolayer. Optional statistical filters have been included to aid in data analysis. This new digital permeability system is able to measure flux rates over a dynamic range from 10^{-9} cm/s/cm H_2O to 10^{-4} cm/s/cm H_2O. Values obtained for cultured lung microvascular L_p are nearly identical to other cultured endothelial monolayers and also to values obtained in-vivo using the Landis–Michel technique and the split–drop method. The use of a commercially available platform allows the system configuration to be easily modified to suit the experimental needs. The technical development of this system is described in detail.
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Introduction

The Michel–Landis technique represented a major technical advancement for measuring hydraulic conductivity (L_p) of microvessels and the continuing use of this method has resulted in a significant contribution to our understanding of microvascular permeability (Michel et al., 1974). With this technique, a capillary or post-capillary venule is cannulated and the vessel can be perfused with solutions of defined composition. When occluded downstream of the cannulation site, a desired hydrostatic and/or oncotic pressure gradient can be imposed across the vessel wall. The continued motion of red blood cells present in the capillary, following occlusion, is assumed to be caused by trans-vascular fluid filtration (J_v). Thus, red blood cells may be used as flow markers and, when tracked over time, allow for the determination of volumetric flux rate (J_v) through the capillary wall. Variations of the Landis method, which are based on tracking the displacement of red blood cells after vessel occlusion but without prior vessel cannulation, have also been developed (Harris et al., 1993; Lee et al., 1971).

In addition to these in-situ techniques, the use of cultured endothelial monolayers has added a complimentary approach toward understanding biochemical and molecular aspects of permeability that are otherwise unavailable in whole vessel preparation. Apparatus to measure L_p of cultured endothelial monolayers has relied on tracking the movement of an air bubble (Dull et al., 1991) or a fluid meniscus (Suttorp et al., 1988) analogous to the red blood cell tracking utilized in the Michel–Landis technique. All of these methods rely on the ability to accurately measure the displacement of a flow marker but require off-line or post-experiment data analysis. The

system developed by Tarbell et al. (1988) and modified by Dull et al. (1991) utilized a technique such that trans-endothelial fluid flux caused the displacement of an air bubble contained in a length of narrow bore glass capillary tubing. The displacement of the bubble was measured and converted into a volumetric flux rate. In the earliest version of this system, displacement of the air bubble was tracked manually with the aid of a microscope and magnetic displacement transducer (Dull et al., 1991) and demonstrated, with appropriate culture conditions, L_p values nearly identical to those values obtained using in-situ preparations (Bhattacharya, 1987; Huxley and Curry, 1985; Kendall and Michel, 1995). This system was automated by developing a light emitting diode (LED) linked to a motorized gear drive that tracked the air bubble–fluid interface (Sill et al., 1995). The tracking mechanism was driven by a stepper motor controlled by a computer such that the bubble displacement, or fluid flux, could be displayed on the computer screen in real time.

While these computerized systems represented technological improvements, there were several limitations that often hindered the experimental plan. In our experience in using this equipment (Chang et al., 2000; Dull et al., 2001), the most difficult aspects of the LED-based apparatus were: 1) the small optical window (approximately 0.5 cm) in which the system could detect the air bubble and 2) the slow response time of the software–motor interface that moves the LED along the capillary tube. The combined effect of these two limitations was a limited dynamic range, preventing measurement at high flux rates and often causing failure to track during flux transients. This required frequent restarts of the software and often important data were missed. To overcome these technical shortcomings, we envisioned a completely new system based on our proven air bubble-tracking paradigm but with the capacity: 1) to measure bubble displacement during periods of rapidly changing flux rates, 2) to be written in a computing environment that would allow easy end-user re-configuration based on unique experimental needs, and 3) for overall greater automation.

Microvascular researchers have adapted commercial programs such as Adobe Premiere for the analysis of video images of the microcirculation (Norman, 2001), developed their own software using traditional programming languages as we have done in the past, and also taken advantage of graphical software design tools such as National Instruments LabVIEW. Davis et al. have recently demonstrated for automated quantification of vessel diameter (Davis, 2005). Whereas our former system was written in C++ and designed to run in a DOS-based computing environment, our new system would be a virtual instrument (VI) written in this commercially available development environment. LabVIEW is used to graphically create custom VIs for laboratory data acquisition and synthesis (Bryant and Gandhi, 2005; Stoll et al., 1996) and has enabled investigators to develop experimental methods that would not be otherwise feasible in terms of time or expense. The LabVIEW development environment has proved to be a robust and flexible tool for the development of laboratory software and can allow a researcher to program new custom functions as fast as an experienced conventional computer programmer (Gregory et al., 1994; Kalkman, 1995). The methodology and software design principals which we demonstrate here can easily be extended to encompass a variety of experimental applications with a minimum of software design experience.

Materials and methods

Permeability system

A combination camera stand and glass capillary tube holder was built as one tightly fitted structure and machined from heavy aluminum. This combination design allows the camera to be calibrated once for multiple uses of the equipment instead of recalibrating whenever the tubing is removed. The digital video feed from a SONY RS-170 monochromatic video camera was acquired through a National Instruments IMAQ PCI-1407 single channel analog video capture board.

Programming of the video tracking and analysis software was done using National Instruments LabVIEW. Where text-based programming environments build and run applications based on languages such as a C++ or Java, LabVIEW uses structured dataflow diagramming to create a visual representation of how data in the application will be handled. The Virtual Instruments (VIs) created can be run within the LabVIEW development environment or can be compiled into stand-alone applications for use on any Windows or Macintosh computer. Both the Data Acquisition and the Data Analysis VIs were created and run in LabVIEW 6.1 with the optional Vision Development Module. Tracking of fixed objects over several hours was performed to ensure that the system was free of drift.

Cell culture

Bovine lung microvascular endothelial cells were purchased from Vec Technologies (Rensselaer, NY) and grown in MCDB-131 Complete (Vec Technologies, Albany, NY). Cells were subcultured onto Snapwell chambers (Costar, polycarbonate membranes, 0.4 u pore size, 12 mm diameter). Prior to seeding the cells onto the membrane, the Snapwell chambers were washed with 0.5% acetic acid for 1 h then gelatinized with 0.2% gelatin for 1 h. Cells were plated at a density of 2.5×10^5 cells/cm^2, and all L_p measurements were conducted 10 days after plating.

L_p experiments

The Snapwell culture unit was inserted into a custom-made permeability chamber that consisted of two pieces of transparent polycarbonate. The bottom half contained a small reservoir (ablumenal reservoir) and a rubber O-ring that fit snugly under the rim of the Snapwell. The top half of the chamber was designed with another O-ring that sealed against the top rim of the Snapwell. Two spring loaded latches on the outside of the chamber pulled the two halves together to compress the O-rings and formed a water tight seal both above and below the Snapwell. The top half of the chamber had a small concavity machined into the roof which created a reservoir above the cells of approximately 1 ml. The top half of the chamber also contained two ports for inflow and outflow of tissue culture media. The inflow port consisted of a three-way stopcock, and the outflow port was a two-way stopcock. The cell culture chambers rested inside a water bath maintained at 37°C.

To measure L_p, the inflow port of the cell culture chamber was connected by silastic tubing to a pressure manifold filled with MCDB-131 (1% BSA, 25 mM HEPES, 1% pen/strep). The air bubble flow marker was tracked as it passed through an 18 cm length of clear borosilicate capillary tubing (Wilmad Glass, Buena, NJ) with an internal diameter of 1.9913 mm ± 0.0004 in. The glass capillary tubing was held in place by a custom-made aluminum plate able to hold up to 6 capillary tubes. A small air bubble was injected into each capillary tube to serve as a flow marker. Once the permeability chamber was fully assembled, a hydrostatic pressure head (ΔP) was set to