A DEVICE FOR STERILE MEASUREMENT OF TRANSEPITHELIAL ELECTRICAL PARAMETERS OF CULTURED CELLS

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SUMMARY: Cultured epithelial cells grow into organized epithelia and when grown on permeable supports they develop distinctive electrical properties. These electrical properties, which include transepithelial potential difference (PD), resistance, and short circuit current (Isc), may be used to characterize the epithelia in terms of organization, transport, and response to hormones. We have developed a simple device for the repetitive sterile measurement of the transepithelial PD and resistance and Isc of epithelial cells grown in filter-bottom cups. The device is basically a two-part open chamber into which the filter-bottom cup is placed, and the electrical seal between apical and basolateral surfaces is formed by the sides of the filter-bottom cup. The chamber is maintained sterile so that repeat measurements may be made in a laminar flow hood over extended periods of time without risk of infection. Measurements of transepithelial PD, resistance, and Isc of A-6 cells or TB6C cells made with this device are not different from the same measures made in a more conventional Ussing-type chamber (which was used to establish the relation between Isc and isotopically measured net Na⁺ flux). Measurements of potential difference are identical to those of a commercially available device, and resistance measurements are similar when appropriate backgrounds are considered. Our device is a simply constructed and easily maintained instrument for monitoring transepithelial electrical characteristics of cultured cells and their responses to hormones or other agents.

Key words: sterile measurement; electrophysiology; short circuit current; potential difference; epithelial cell; porous-bottom culture dish.

INTRODUCTION

Cultured epithelial cells are now widely used in the study of many aspects of epithelial structure and function including the development and maintenance of epithelial polarity, formation of tight junctions, and the development and regulation of cellular and transepithelial transport. To take full advantage of cell culture systems for such studies, it is often necessary to grow the cells on some form of porous-bottom culture dish to better simulate physiologic conditions and to allow access to both sides of the polarized epithelium. It is often useful under such circumstances to measure transepithelial electrical characteristics such as potential difference, resistance, and short-circuit current. We have developed a simple device for the repetitive, sterile measurement of transepithelial electrical characteristics for use with cultured cells grown on porous-bottom dishes. In this paper we describe the construction and use of this device and provide comparisons to electrical measurements made in more conventional Ussing-type chambers and with currently available commercial devices.

MATERIALS AND METHODS

The basic device consists of a two-part chamber as illustrated in Fig. 1. The base of the chamber contains a well into which the porous-bottom culture dish is placed. The bottom of the well is filled with medium or Ringer's solution and contains two ports which are tunneled from underneath the porous-bottom culture dish to better simulate physiologic conditions and to allow access to both sides of the polarized epithelium. It is often useful under such circumstances to measure transepithelial electrical characteristics such as potential difference, resistance, and short-circuit current. We have developed a simple device for the repetitive, sterile measurement of transepithelial electrical characteristics for use with cultured cells grown on porous-bottom dishes. In this paper we describe the construction and use of this device and provide comparisons to electrical measurements made in more conventional Ussing-type chambers and with currently available commercial devices.
chamber rests on the lower flange all bridges are properly placed. Both chamber top and bottom are made from a solid rod of polycarbonate.

The chamber top contains three holes around the outside which are equal to the diameter of the middle section of the stainless steel rods coming from the base. The rods serve then to guide the top into the proper position for bridge alignment and allow the top to rest in an up or down position. Four additional holes are drilled in the top, two to receive small "towers" containing calomel half-cells for measurement of voltage, and two to receive small towers containing Ag-AgCl wires for passing current. The construction of the towers is described below. The center two towers contain the calomel half-cell and the Ag-AgCl wire for voltage measurement and current flow to the apical solution and are aligned to be directly above the tunneled ports in the base of the well. The lateral or outer two towers contain the calomel half-cell and the Ag-AgCl wire, which align with the tunneled ports on the lateral aspect of the chamber bottom and which connect to the basolateral solution. The bottom of each of these four holes for the towers is threaded to receive a threaded guide containing a standard KCl-Agar bridge. When screwed in place the threaded guide compresses an O-ring so that it seals against the bridge. The bridges are cut so that the potential bridge terminates 2 to 3 mm from the surface of the cells, and the current bridge terminates 1 cm from the surface of the cells when the chamber top is lowered.

The towers are constructed from 3-cm lengths of glass tubing, the upper end of which is fire-polished to a narrow opening to receive either a platinum wire (for the calomels) or a short length of silver wire which has been coiled and chlorided. The wires are secured into the glass tubing by a small amount of Epoxy and supported mechanically by a snugly fitting piece of silicone rubber tubing. The free end of the wires protruding outside the silicone rubber tubing can then be soldered to copper wires for connection to any voltmeter and external current source, such as a commercial voltage clamp. The calomel half-cells are constructed in the towers containing the platinum wires. With the tower held upside down so that the wire is at the bottom, the wire is covered with a drop of clean elemental mercury. This is then covered with a 1-mm layer of calomel made by mixing mercurous chloride, mercury, and saturated KCl in a mortar and pestle. This is secured in place by gently packing with a small plug made from glass-wool soaked with saturated KCl. The bottom of the glass tubing is then fitted with Tygon tubing, which is plugged snugly into the appropriate hole on the upper surface of the chamber top. The top is then turned over and the towers and connecting spaces are filled with 3 M KCl through the threaded

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FIG. 1. In-Hood short circuiting device. Left, the base of the unit with a filter-bottom cup in place. 1, double-flanged steel rods for guiding the upper chamber; 2, tunneled ports for the bridges to the basolateral solution. These ports are tunneled to the outlets of the base unit (3, center). The top unit holds calomel half-cells and silver wires in short "towers" for measuring potential difference and passing current. Central towers (4) are for the apical solution, outside towers (5) are for the basolateral solution. Connection to solutions is made via KCl bridges (7). Holes are drilled for guiding steel rods (6). Right, top lowered for making measurement of PD and current.