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Journal of Cystic Fibrosis 3 (2004) 123–126
Journal of Cystic Fibrosis
www.elsevier.com/locate/jcf

Transepithelial electrical measurements with the Ussing chamber

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Available online 2 July 2004

Abstract

The Ussing chamber technique is a simple, but powerful technique to investigate ion transport. Originally designed to study vectorial ion transport through the frog skin, it has revolutionized our knowledge about how electrolytes permeate epithelia. Here we discuss the physiological principles that underlie the technique and protocols to investigate the role of the cystic fibrosis transmembrane conductance regulator (CFTR) in transepithelial ion transport.

Keywords: Epithelial ion transport; Transepithelial resistance; Transepithelial voltage; Short-circuit current

1. Introduction

When Hans Ussing invented the device that was accordingly named after him "Ussing chamber", he could never have anticipated the broad range of applications that his system would be used for [1]. The Ussing chamber consists of two functional halves. One is the chamber itself and the other the electrical circuitry. To this end, a plethora of technical variations for both of these subunits has been developed. Chambers come in all kinds of different sizes and shapes, while the electronic circuitry allows not only the measurement of resistance (R), current (I) and voltage (V), but also complex parameters including impedance and capacitance. In this article, we will review the basic principle of the technique and the most common applications for Ussing chamber measurements.

2. Two different types of chambers

There are currently two types of Ussing chambers: the circulating chamber and the continuously perfused chamber. Fig. 1 shows a schematic representation of a circulating chamber.

The circulating chamber consists of a U-shaped tubing system usually made of glass that is filled with the experimental solution. The tubing can be heated if required and is gassed either with air or other gases such as CO_2, O_2 or N_2. The gassing serves two purposes: first, to oxygenate the liquid contents and, second, to stir the liquid to ensure complete convection (termed "bubble lift"). The U-shaped tube secures an equal hydrostatic pressure on both sides of the chamber and, thus, avoids damage caused by bending of the tissue. During the course of an experiment, substances are usually added to one or both sides of the tube in a sequential manner. It is obvious that once added, all substances persist in the solution until the end of the experiment. Because most experiments do not require a control recording after drug treatment, the circulating chamber has proved to be fairly robust and simple to use.

The continuously perfused chamber is not yet commercially available, but it can be manufactured with the help of qualified machine shops. Fig. 2 highlights the features of this design. The two half-chambers are designed to minimise the hydrostatic pressure and, thus, prevent serious damage to the tissue during perfusion. The solutions bathing the two sides of the tissue are delivered to the chamber from reservoirs mounted 20–50 cm above the chamber via polyethylene tubes into the chamber. Valves may be used to regulate the flow rate, which is otherwise dependent on the diameter of the tubes and the hydrostatic

Abbreviations: I, current; R, resistance; R_a, resistance of the apical membrane; R_b, resistance of the basolateral membrane; R_t, transepithelial resistancey?; V_{te}, transepithelial voltage; V, voltage; V_a, voltage of the apical membrane; V_b, voltage of the basolateral membrane.

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doi:10.1016/j.jcf.2004.05.026

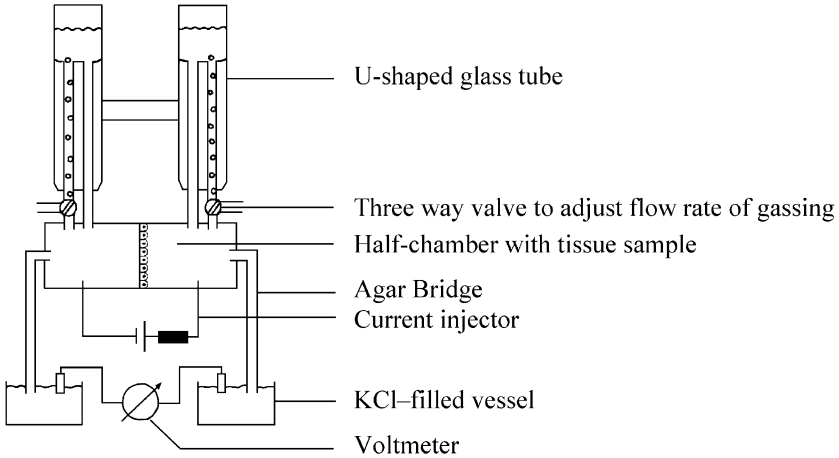

Fig. 1. Schematic drawing of a circulating Ussing chamber.

pressure. Temperature can be adjusted by means of a water jacket heating system.

Most chambers are usually made of Teflon or Lucite and come in numerous sizes and shapes. A very new approach has been chosen in one of the author's former lab in Freiburg.[1] For the use of very small tissue samples such as respiratory airway and colonic biopsies (diameter <1 mm), a small circular disk that holds the tissue is inserted between the two half-cells once everything is prepared [2]. This approach has proven to be very successful and is becoming widely adopted by labs around the world.

Many labs wish to use cultured cells grown on permeable supports. In fact, if one studies the literature this seems to be the widest application of the Ussing chamber nowadays. In this case, one is well advised to use commercially available products. Further information can be found in an accompanying article [3]. Very often, the same company that manufactures the permeable supports also offers matching Ussing chamber half-cells.

3. Resistance

Epithelia display two features that distinguish them from all other tissues: polarity and tightness. Polarity is generated by the asymmetric distribution of proteins to either the apical or the basolateral side of the cellular membrane. An assembly of proteins called "tight junctions" separates both sides of the membrane, while sealing adjacent cells. The formation and permeability of tight junctions determines the resistance and integrity of the tissue. The resistance (R) is given by $R=\rho l/A$ where ρ is the specific resistance module of the material, l the length or thickness of the material (constant for each tissue preparation) and A the area. Considering a tissue, R can be broken down into an arrangement of resistors. As can be deduced from Fig. 3, we can model the electrical parameters in a layer of cells using a series of two resistors, R_a (resistance of the apical membrane) and R_b (resistance of the basolateral membrane), shunted by a parallel resistor, R_{Shunt}.

The total transepithelial resistance (R_t) is defined by Kirchhoff's law as:

$$R_t = \frac{(R_a + R_b)\cdot R_{Shunt}}{R_a + R_b + R_{Shunt}}. \quad (1)$$

It can easily be anticipated that in a real experiment, the crucial determinant for R_t will be the integrity of the tissue and, thus, R_{Shunt}. Both improper treatment of the tissue and inappropriate design of the chamber will influence R_{Shunt} significantly, eventually rendering electrical measurements impossible.

What is the proper way to determine R_t? In essence, R_t can be calculated using Ohm's law:

$$R_t = \frac{\Delta V}{\Delta I} \quad (2)$$

The simplest way is to apply a voltage and measure the resulting change in current, an approach that has generally been called "voltage clamping". It is also possible to use an alternative approach called "current-clamp". Here short current pulses are injected via a resistor. The device that measures the voltage deflection is a high impedance (>10 MΩ) voltmeter, while the device applied to the short-circuit technique is a feedback-driven current injector connected to an amperemeter. The measured signal here is a current that is internally "translated" into a voltage. The output signal can then be visualized and recorded by a data acquisition system. Most commercial systems provide both current- and voltage-clamp modes of operation.

4. Transepithelial voltage

Epithelial tissues transport ions and thus, generate a transepithelial voltage, V_{te}. This voltage has been termed

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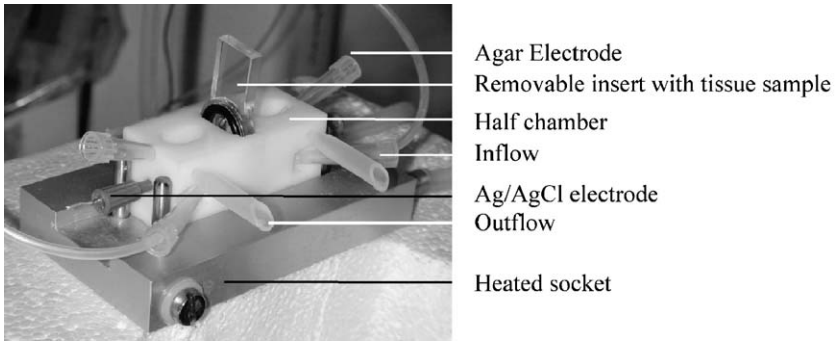

Fig. 2. Continuously perfused Ussing chamber.

accordingly "active transport potential" [4,5]. A prerequisite for such a transport potential is the asymmetric distribution of ion channels on the apical and basolateral membranes of epithelial cells. The net movement of negative or positive charges from the apical to the basolateral side generates a voltage that is equal to the difference between the voltage of the apical membrane (V_a) and of the basolateral membrane (V_b), respectively. Measurements of V_{te} are performed in current-clamp and often referred to as open-circuit recordings. They are useful in studying absorptive as well as secretory mechanisms. In absorptive tissues, V_a is predominantly influenced by the activity of Na^+ channels, whereas in secretory epithelia, V_a is determined by a Cl^- conductance, which most often is the cystic fibrosis transmembrane conductor regulator (CFTR).

5. Short-circuit current

The short-circuit current (I_{sc}) is defined as the charge flow per time when the tissue is short-circuited (i.e. V_{te} is clamped to 0 mV). To measure I_{sc}, a current that is adjusted by a feedback amplifier to keep V_{te} at 0 mV is injected across the epithelium. The amount of current required is continuously adjusted and measured. Intermittently the voltage is clamped to values different to 0 mV thus enabling an estimate of R_{te}. I_{sc} is also given by the equation

$$I_{sc} = \frac{V_{te}}{R_{te}} \quad (3)$$

From this simple equation, it is apparent that I_{sc} can also be calculated under open-circuit conditions when R and V_{te} are known. This value is often referred to in the literature as "equivalent short-circuit", I_{sc} [2].

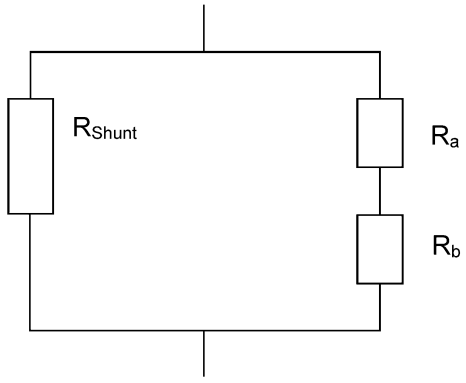

Fig. 3. Lumped model of the equivalent circuit of a tissue consisting of a single monolayer of cells. The total resistance R_t is a function of the parallel assembly of R_{Shunt} and the two series resistors R_a and R_b, respectively.

6. Measurement of apical CFTR Cl^- currents in polarised epithelia by permeabilisation of the basolateral membrane

Because epithelia are composed of apical and basolateral membranes arranged in series, it is difficult to distinguish the function of CFTR in the apical membrane from the activity of ion channels and transporters in the basolateral membrane. One approach to circumvent this problem is to use ion substitutions and drugs that inhibit the activity of different transport proteins. However, a drawback with this approach is the lack of specificity of many inhibitors of transport proteins. An alternative approach is to functionally eliminate the electrical properties of the basolateral membrane by permeabilising the membrane with an ionophore (e.g. nystatin [6]).

Nystatin is a polyene antibiotic that creates aqueous pores in artificial and biological membranes [7]. These pores are 0.8 nm in diameter, large enough to allow monovalent cations, water and small non-electrolytes to permeate. Nystatin pores may also be permeable to anions, such as Cl^-, but the selectivity for anions is reduced compared with cations ($P_{Cation}/P_{Cl} = 9{:}1$ [7]). Importantly, divalent cations and larger molecules, such as ATP, are too large to pass through nystatin pores. To manipulate the ATP concentration within an epithelium, it is necessary to use *S. aureus* α toxin that produces larger pores permeable to ATP [8].

Following the permeabilisation of the basolateral membrane with nystatin, the Ussing chamber technique can be employed to study the function of CFTR in the apical

membrane. This technique has been used to study endogenous Cl^- channels in the apical membrane of airway and intestinal epithelia [9]. It has also been employed to investigate the function of wild-type and mutant CFTR Cl^- channels heterologously expressed in Fischer rat thyroid (FRT) epithelia [10]. For further information about measurements of apical membrane CFTR Cl^- currents, see Ref. [6].

7. How to perform an experiment

The procedure how to conduct an Ussing chamber experiment can be described briefly: once the chambers and solutions are prepared, one is well advised to put everything together without any tissue and flush the system with bath solution. If the system is watertight, the temperature should be adjusted to the desired value. Then the current and voltage electrodes are inserted into the half-cells. Depending on the type of electrode (KCl-filled glass column, agar-bridge, calomel electrode) the resistance of the individual electrode may vary over time, which in turn can cause asymmetries. Therefore, the system should be checked for noise and offset voltages as soon as the electrodes are connected to the current/voltage pulse injectors and the volt-/amperemeter, respectively. By turning on the current/voltage pulses, one can estimate the resistance of the empty chambers, a parameter required for proper calculation of resistance and currents. Some devices offer the possibility to cancel out both the resistance of the solution and the offset voltage generated by unequilibrated electrodes. This procedure should be performed before inserting the tissue or filter. Now the chambers are disconnected from the supply of solution and the tissue mounted. After reassembly of the system the recording can begin. It is a known fact that immediately after tissue insertion, the values of all electrical parameters (V_{te}, I_{sc}, R_{te}) tend to oscillate. This is most often caused by the mechanical stress imposed on the sample or residual stimulation. Therefore, one is well advised to allow the tissue some 10–40 min to recover before beginning experimental manoeuvres. After a stable baseline is reached, the system for data acquisition can be switched to a higher time resolution and the "real" experimental can begin. For further details, see Ref. [11].

8. Conclusion

The Ussing chamber is a very powerful technique to study ion transport across tissues. However, it is a somewhat simple technique to study a very complicated system, a fact often disregarded. Only proper understanding of the underlying physiological principles and their impact on measurements allows researchers to draw the right conclusions.

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