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Multi-electrode system for measurement of transmembrane ion-fluxes through living epithelial cells

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ABSTRACT

Cystic Fibrosis (CF) is the most common fatal human genetic disease. It is caused by the defect in a single anion channel protein which affects ion and water transport across the epithelial tissue. A flat multi-electrode platform of diameter 12 mm, allowing for measurement of four ions: sodium, potassium, hydrogen and chloride by exchangeable/replaceable ion-selective electrodes is described. The measurement is possible owing to the architecture of the platform which accommodates all the electrodes and inlets/outlets. The platform fits to the cup and operates in a small volume of the solution bathing the living epithelial cell layer (membrane) deposited on a porous support of the cup, which allows for effective monitoring of ion concentration changes. By applying two multi-electrode platforms, it is possible to measure the ion transmembrane fluxes. The inlet and outlet tubes in the platforms allow for on-fly change of the calibrants, ion-concentration changes and ion channel blockers. Using different ion-concentration gradients and blockers of ion-transporting molecules we show for the first time that sodium ions flow from the basolateral to apical face of the cell monolayer via a paracellular route and return also via a transcellular one, while chloride anions are transported back and forth exclusively via a transcellular route.

1. Introduction

Epithelial tissues line all the wet surfaces of the body including the lungs, digestive tract, excretory and reproductive systems. Their major function is to directionally transport nutrients, secrete enzymes, hormones and regulate water transport. Epithelial tissue is made of a single layer of cells bound together by tight junctions. The layer forms a barrier to simple water and ion diffusion. Water is transported across the epithelial cell layer by means of osmosis. Electroneutral transport of a single cation accompanied by a single anion causes the passive osmotic flow of (about) 370 water molecules across the epithelium. If the anion or cation transport route is impaired, then there is no electroneutral transport of ions and no water can flow across the epithelial cell layer. Such a defect of the epithelial transport leads to serious human diseases, e.g. cystic fibrosis (CF). CF is caused by the defect in a single gene which encodes an anion channel present in the apical face of the epithelial cell layer, called the Cystic Fibrosis Transport Regulator (CFTR).

For years, scientists have argued whether it is the decreased secretion or enhanced absorption of water that is responsible for viscous apical mucus secretion [1,2]. There is, though, another idea concerning the dense mucus production in CF. While CFTR is called the chloride channel, it is in fact an anion channel, with a low selectivity coefficient $Cl^-/HCO_3^- = 4$ [3]. Quinton suggested that in CF bicarbonate secretion is affected [4,5]. Measuring the transport of ions and water across the epithelium is thus important to understand the mechanism of cystic fibrosis.

Ion-transporting molecules present on both sides of human bronchial epithelium cells were reviewed previously [6–9]. Ion transport across the human bronchial epithelium involves five types of ions (sodium, potassium, proton, chloride and bicarbonate) which move in both directions at the same time and reach a new equilibrium within a few minutes. Appropriate for the purpose of monitoring ion transport across epithelial cells is the application of ion-selective electrodes (ISEs). Ion-selective electrodes are electrochemical sensors widely used in the biomedical, environmental or industrial fields. The key application area of ISEs is in clinical chemistry, where routine measurements that are fast, inexpensive, reliable and service-free, and fully automated are of importance [10,11]. Today, ISEs are widely and routinely used for the determination of electrolytes (K^+, Na^+, Cl^-) and pH in physiological fluids such as whole blood, serum, plasma or urine. The ISEs used in routine clinical analysis are characterized by their long lifetime (typically at least 10,000 segmented samples of blood), the low sample volume needed for

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measurement (less than 30 μl), the stability of the measured signal (low drift and stable standard potential owing to conventional ISE design with internal solution or gel) and their fast response to ion concentration changes (a few seconds due to high instrument capacity demand) [10,11]. To avoid extra costs, the ISEs are used in the form of flow-through electrode blocks which allow service to replace a single electrode when it malfunctions.

It is not recognized that these features of ISEs outlined above promote the electrodes for use in other important biomedical measurements, for instance, for the measurement of ion transport through living epithelial cells as described herein. In this instance, the flow-through electrodes must be placed on one flat surface (platform) which constitutes a probe that can fit the cup with epithelial cells (e.g. Snapwell) and work in proximity of the cells' surface, about 100 μm. The platform should ensure that the measurements take place in a small volume of solution (about 10 μl) that bathes the cells for over few hours of continuous measurement. Preferably, as in the case of routine clinical measurement, the replaceability of a single electrode would be advantageous, in this case to avoid the need for a whole probe change. A multi-electrode platform with solid-contact electrodes has recently been reported for measurements of ions in a blood drop [12], and the multi-electrode system without the possibility of a single electrode replacement for ion fluxes in the epithelial layer was described by our groups [13]. Both platforms were not fully satisfactory for long-lasting monitoring, the former because of unstable standard potentials induced by solid-contacts, and the latter because of the excessive need to substitute the whole probe when one electrode readout is erratic.

In this report, we show the advanced 3D architecture re-design of the ISE block which is achieved by preserving the crucial materials, electrochemical and design features known from routine clinical chemistry [10]. Namely, instead of acrylic flow-through ISEs and reference electrode, we have engineered a 12 mm diameter flat platform able to accommodate 4 ISEs, reference electrode and inlet/outlet tubes. We significantly advance our previous study [13] by using new ion blockers of sodium and chloride channels, by applying deliberate sodium and chloride gradients, and by making the ion-selective electrodes in the platform replaceable. In this way, we are able for the first time to report credibly on sodium and chloride transport pathways as well as make new inroads into gaining information on the role of bicarbonates in ion fluxes through epithelial cells.

2. Methods

2.1. Micro-fabrication of the electrodes

The 16 mm long micro-electrode body was made of commercially available 3 mm diameter PMMA poly (methyl methacrylate) rods. The outer M3 thread was made to fit the electrode into the measuring platform. A 1 mm bit was used to drill through the body. Then from the opposite side to the M3 thread, the inside bore was widened by a 1.5 mm bit to a depth of 12 mm, and the inner M2 4 mm long thread was then cut in the electrode body, as shown in Fig. 1C. Commercially available M2 PE (polyethylene) screws were drilled through by 0.6 mm bit, and 0.6 mm diameter, 99.9% Ag pure silver wire was pushed through the PE screw - Fig. 1C. The M3 thread was made on the inlet and outlet 3 mm outer diameter steel tubes which served as the fluid outlet and inlet. The measuring platform was made of 12 mm diameter PMMA rod which was drilled by a 10 mm bit leaving a 3 mm thick support in which seven M3 threaded orifices were made to hold four ion-selective electrodes, one reference electrode and two inlet and outlet tubes. The micro-machined electrodes and the measuring platform are shown in Fig. 1.

2.2. Silver/silver chloride internal reference electrodes

The silver wire was mounted in the PE screws and was then coated with silver chloride. Electrolytically cleaned silver wire served as the anode and platinum wire as a cathode. Both were placed in 100 mM KCl solution and a current of 0.1 mA/cm^2 was passed through the system for 2 h.

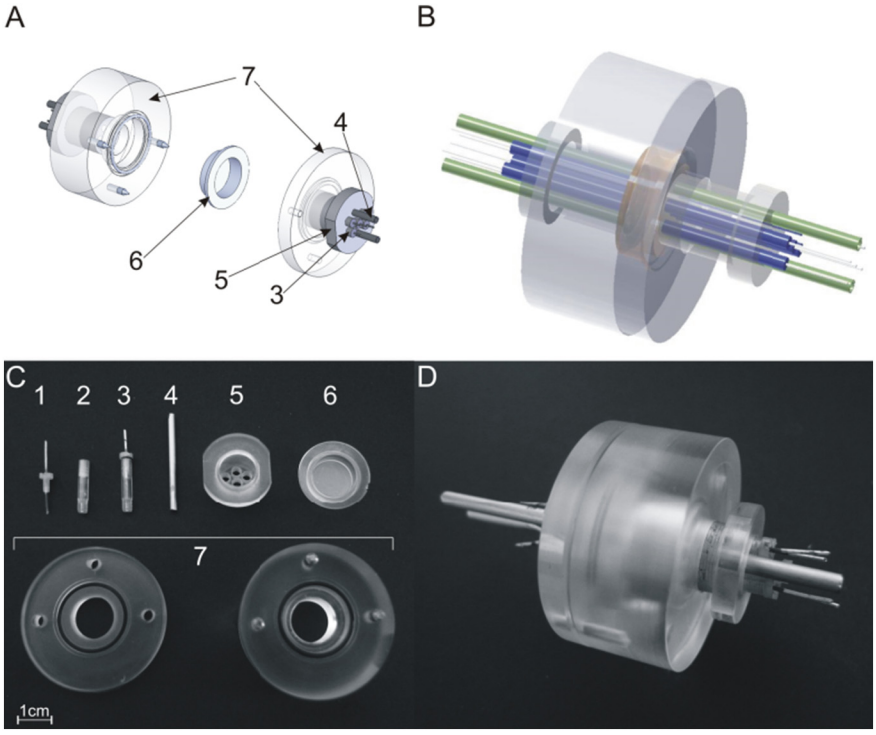

Fig. 1. Micro-electrodes and the measuring platform. A. Design graphic of unmounted measurement system. B. Design graphic of mounted measurement system. C. Real picture of measurement system elements (1: silver/silver chloride wire in M2 PE screw, 2: micro-electrode body, 3: mounted micro-electrode, 4: steel tube - solution inlet/outlet, 5: microelectrodes' holding probe, 6: Snapwell cup, 7: external probe holders. D. Real picture of mounted measuring system.