Ussing chamber

Classical type with improved specifications

General description

Originally this chamber was designed for the investigation of ion transport through intact epithelial tissues obtained from animals (frog skin, toad urinary bladder, rabbit colon, etc…) [1]. Tissues were glued on a Lucite ring with glue used in surgery (hystoacryl blue) and mounted between the two chamber halves. This Lucite ring with tissue fits around the rim (width 2 mm) that delineates the inner area of the chamber. The glued parts of the tissue are not in contact with the perfusion solutions in the chamber. Silicon grease that is spread out over the rim prevents leaks and guarantees tight sealing without edges damage. Alternatively, thin disks with a hole of 2 mm have been used to mount tissues with relative small area as the intestine of the mouse [2]. The introduction of the ring with tissue is illustrated in Fig. 1. The chamber is particular useful for the investigation of monolayers of polarized cultured cells grown on permeable supports (Nunc anopore membranes, Costar, Millipore, etc…).

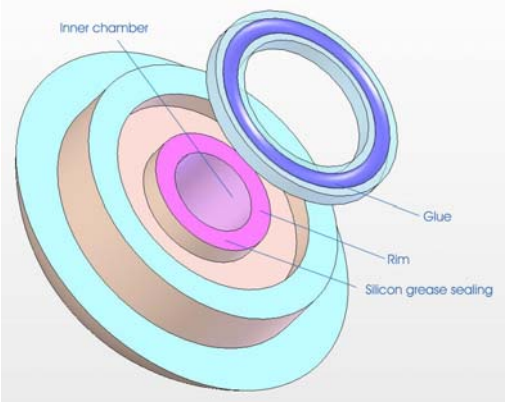

Figure 1: *Mounting of tissue on ring and fitting into chamber.*

The chamber has been designed for experiments where continuous perfusion is required as well as for protocols where stopped flow is needed. Continuous perfusion is generally preferred to keep stable and constant exposure conditions to the cells. In continuous perfusion mode, the solutions can be recycled with a peristaltic pump system, or, solution outflow by gravity is possible in circumstances where noise sources and mechanical vibrations have to be minimized (e. g. when performing the analysis of the fluctuation in transepithelial current – noise analysis). However, in cases where for instance transepithelial flux of dyes or markers are studied, stopped flow conditions might be required. Under the latter conditions, mixing of the solutions in each compartment is possible with a small magnetic stirring bar that is kept in rotation by magnets mounted on a motor that is installed in the support of the chamber. In stopped flow conditions, an O_2-CO_2 gas bubbling device can be installed from the top of the chamber.

1 - I. De Wolf & W. Van Driessche, W. Nagel: Forskolin activates gated Cl channels in frog skin. *Am. J. Physiol*., (1989) 256, C1239-C1249.
2 - E. Ghanem, B. Robaye, T. Leal, J. Leipziger, W. Van Driessche, R. Beauwens and J.-M. Boeynaems: The role of epithelial $P2Y_2$ and $P2Y_4$ receptors in the regulation of intestinal chloride secretion. *British Journal of Pharmacology*, (2005) 146: 364−369
3 - M. Stockmann, A. H. Gitter, D. Sorgenfrei, M. Fromm and J. D. Schulzke: Low edge damage container insert that adjusts intestinal forceps biopsies into Ussing chamber systems. *Pflügers Archiv*, (1999) 438: 107-112.
4 - S.P. Srinivas, M. Satpathy, P. Gallagher, E. Lariviere, W. Van Driessche: Adenosine induces dephosphorylation of myosin II regulatory light chain in cultured bovine corneal endothelial cells. *Exp. Eye Res*., (2004) 79: 543-551

Specifications

- Tissue types used with the chamber.
  - Native tissues of large size from animals: colon, intestine, skin, bladder, etc…
    Tissues are glued with basolateral side to a Lucite ring (see Fig. 1).
    Dimensions of ring: ID: 13 mm, OD: 18 mm, Thickness: 1.5 mm.
  - Monolayers of cultured epithelial cells grown on permeable membranes.
    For instance: - A6 epithelia cultured on Nunc anopore filters,
    - MDCK cells cultured on Costar transwells.
  - Monolayers of endothelial cells cultured on permeable supports, see ref. 4.
  - Small area preparations: tissue from mice (intestine) or biopsies.
    1 - Tissues are glued to a thin ring with small opening.
    For mounting method, see references 2 and 3.
    2 – Alternatively, an adapter that holds the tissue can be mounted in the orifice of the chamber.
- Edge damage is prevented by minimizing the compression of the tissue. This is achieved by leaving a narrow space between the rims near the orifice of the chamber. The width of the space is determined by the thickness of the separation ring (see yellow ring in Fig. 2). The selection of the ring thickness depends on the type of preparation. Silicon grease is used to prevent leaks. With tight epithelia used in non-transporting conditions, transepithelial resistances of 20-30 kOhm are generally obtained.
- Membrane area exposed to the solutions: 0.5 cm^2 (8 mm diameter).
- Volume: each chamber half has a volume of 1 to 1.5 ml.
- Voltage electrodes.
  - Agar bridge in PE tubing of 2 mm OD.
  - Agar bridge fits in Lucite holder filled with KCl solution.
  - Connection with electronic equipment: Ag/AgCl wire.
  - Distance of tip of electrode from membrane surface: 1 mm.
  - Resistance is generally in the range of 3 to 5 kOhm.
- Current electrodes.
  - Construction: similar to voltage electrodes.
  - Distance of tip of the electrode from membrane surface: 16 mm.
- Continuous perfusion of each compartment. Perfusion by gravity is recommended.
  Inflow occurs through a fine needle. The incoming solution enters the chamber perpendicular to the epithelium and hits the membrane, which prevents unstirred layers and rapid exchange of the solution composition near the membrane surface.
  Outflow: (a) removal of solution by (peristaltic) suction pumps.
  Inexpensive peristaltic pumps are available at EP-Devices.
  The use of pumps enables easy recirculation of the perfusates.
  (b) Outflow by gravity is possible through the outlet tube that is provided with the chamber.
- Stop flow application (optional feature).
  Stirring of solutions is provided by a magnetic stirring bar that is coupled to a magnet that is located underneath the chamber. The magnet is rotated with variable speed with a DC motor. This feature is optional.
  CO_2 and O_2 supply is achieved with a bubbling device that is mounted on the open orifice of the chamber.
- Closing the chamber assembly.
  After the tissue or filter with the monolayer of cells is introduced into one chamber halve, the two parts are brought together. Alignment of the two halves is achieved with a pin and hole arrangement. The chamber halves are held together with stainless bars (3 mm diameter) and nylon nuts. Stainless steel bars slide into the grooves on the side of the chamber assembly. This way of assembling avoids that nuts have to be removed from the bars. The materials used are non-corrosive.

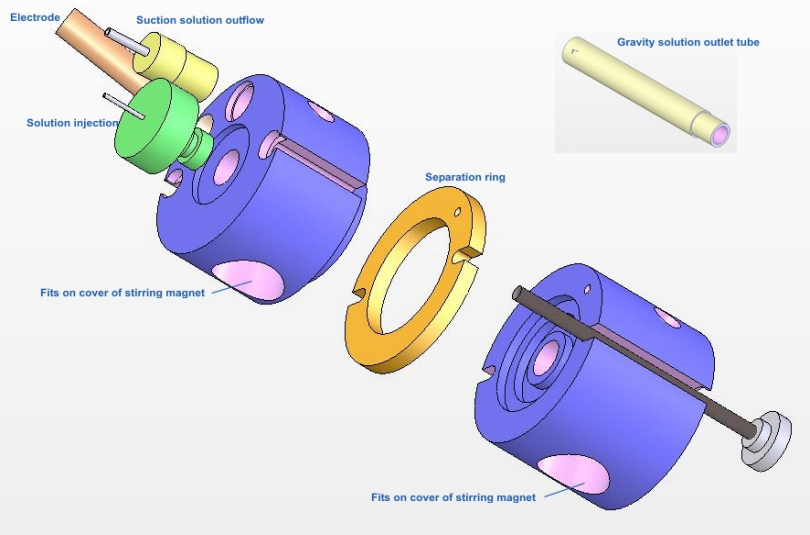

Figure 2: *Open view of chamber assembly.*

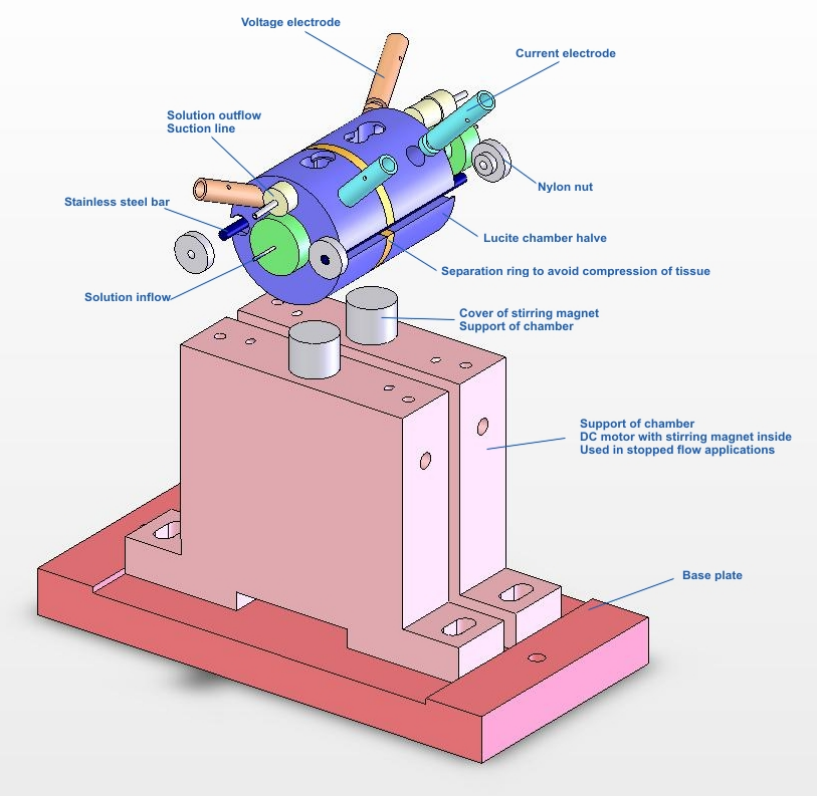

Figure 3: *Sketch of assembly of chamber and support. A DC motor with variable speed on which a magnet is mounted can be installed in the left and right part of the support. The use of this stirring device is required to obtain rapid washout of tracers and markers from the epithelial cells in conditions where the chamber perfusion is interrupted.*