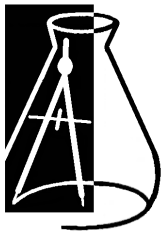

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Innovative Engineering for Science

MEMBRANE CHAMBER
MC

BRAIN SLICE CHAMBER

CAUTION !

YOUR BRAIN SLICE CHAMBER IS A PRECISION ENGINEERED TOOL FOR SCIENTIFIC INVESTIGATIONS. PLEASE TAKE A FEW MINUTES TO FAMILIARISE YOURSELF WITH THE CHAMBER AND READ THROUGH THIS SHORT MANUAL BEFORE ATTEMPTING TO USE THE SYSTEM.

DO NOT USE ALCOHOL OR SIMILAR SOLVENTS IN ANY CONCENTRATION ON ANY PART OF THE CHAMBER SINCE AS WITH MOST ACRYLICS, ™PERSPEX MAY FRAGMENT OR DEVELOP HAIR-LINE CRACKS.

CONTENTS **SECTION**

I

CHAMBER DESCRIPTION

The MC slice chamber is designed to maintain isolated, living tissues *in vitro* and allow stable electrophysiological recordings to be made from the preparation. Brain slices are submerged on to a completely flat, transparent semi-permeable membrane. This offers ideal conditions for both inverted and upright microscopy of *in vitro* preparations.

CONSTRUCTION

The chamber is machined from a single disc of acrylic with a diameter of 101.6mm (4 inches) and an overall height of 25mm. Incubating media enters from the side into a buffer well and then into a cavity formed by a glass cover slip at the base and a stretched semi-permeable membrane attached to an acrylic ring at the upper end. Brain slices are placed submerged on to the flat membrane surface. The utilisation of a membrane with a high molecular weight cut-off ensures all nutrients and oxygen can flow freely for optimum perfusion. The design of the flow path is such that rates up to 20ml/min can be used without inducing mechanical noise. A further advantage is that the high speed flow of oxygenated aCSF directly underneath and across the semi-permeable membrane exerts a Bernoulli effect, resulting in a pressure difference between the upper and lower surfaces of the membrane. This in turn causes a significant movement of nutrients and oxygen downwards through the semi-permeable membrane, increasing the availability to the slice especially on the underside and thereby surpassing passive slice perfusion techniques. The active flow downward through the membrane keeps the slice from floating and makes it mechanically stable. The transparent membrane offers ideal conditions for imaging through a glass cover slip window on the underside of the chamber with an inverted microscope whilst the upper chamber is exposed for microelectrode access. Alternatively there is sufficient access from above for upright microscopes to allow immersion objectives to be used and allow access with microelectrodes from the front and sides. Incubating media leaves the chamber via an exit well, interconnected via a 'U' trap to a second well, in which a steel tube is used to aspirate excess solution and also adjust fluid height in the chamber. The 'U' trap prevents mechanical interference caused by the aspiration process from reaching the slice recording area.

II

METHOD OF PERFUSION

The perfusion solution enters from a side port on the chamber and leads into a buffer well designed to smooth the inflow and release any excess oxygen that has come out of solution before entering the cavity below the semi-permeable membrane. A piece of coarse nylon mesh or other inert material is loosely pushed into the buffer well. This reduces inflowing turbulence by offering resistance and also forms a high surface-to-volume ratio substrate on which excess gas bubbles coalesce and float out of solution. Perfusion solution may be pre-heated to the required temperature before entering the chamber supplied from an external reservoir. An in-line solution heating system (MH03 currently under development) can be used to provide temperature controlled liquid to the side port.

A through-flow from the side port to the cavity and ultimately the exit well needs to be established and any bubbles caught in the flow path are removed by applying a plastic pipette tip to the exit port adjacent to the membrane disc and pumping a few times. A continuous flow of solution during this pumping action ensures that all bubbles are dispelled from the cavity. The exit steel tube height can be adjusted to aspirate to a level that keeps the slice completely submerged. Setting fluid height will be unstable when the chamber system is new or has been cleaned recently due to the nature of polished acrylic surfaces which are hydrophobic. It often helps to leave aCSF solution (without glucose) filled in the chamber for a period over-night. Acrylic plastic has a very small content of water in the structure and allowing salts to adhere to the surface renders it hydrophilic (negative meniscus forms easily) and this aids stable fluid level control.

III

OXYGENATION

Oxygen is supplied to the slice preparation entirely through the perfusion fluid which should be pre-gassed prior to entering the chamber. Saturation with oxygen should be done at close to the final temperature that will be used at the recording site. This ensures that oxygen does not come out of solution when reaching a higher temperature close to the chamber. For example cold stock solution will hold more oxygen in solution: when it reaches a warmer chamber, the oxygen will be liberated out of solution and can give rise to bubble formation in the chamber cavity and interfere with the optics.

IV

TEMPERATURE

IN-LINE SOLUTION HEATER MH03 AND PROPORTIONAL TEMPERATURE CONTROLLER PTC03

DESCRIPTION

An in-line solution heating system similar to MH02 is currently under development specifically for the MC. This will be able to support heated perfusion solution flow at 20ml/min.

SPECIFICATIONS OF MH03 (under development)

SPECIFICATIONS OF PTC03

Readout accuracy	+/ - 0.1 degrees centigrade
Control accuracy	0.5^{o}C below set temperature maximum difference.
Control stability	Not more than +/ - 0.1^{o}C from control point.
Output power	36 Watts Max.
Output type	D.C. Proportional control
Sensors	Pt100 Platinum Resistance (Control & Monitor)
Power requirements	110V / 240V +/ - 10% 60/50Hz, 50 W (specified on order).
Dimensions mm	90H x 260W x 260D
Weight	4 Kg

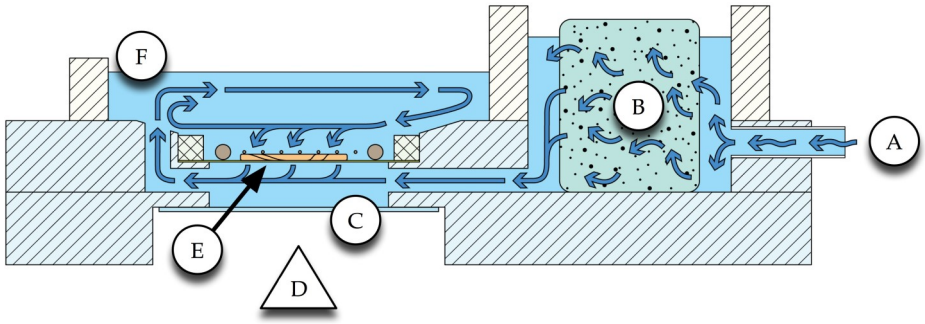
INSTALLATION AND OPERATION

Your parcel should contain the MC together with the following:

1) An 'L' shaped steel tube that locates with the slit-end into the exit well when positioned in the clamp.
2) A set of acrylic rings for attaching the semi-permeable membrane.
3) 'C' Clamp for holding down finished membrane discs into chamber.
4) Acrylic former and rubber 'O' ring kit to make new membranes.
5) Nylon mesh pad for forming buffer in input well.
6) Pin tool for extracting membrane discs and 'C' clamp
7) Suppliers of dialysis membrane are listed at the end of this manual

LABEL	**FUNCTION**
[A]	Port connected to aCSF supply
[B]	Buffer material to smooth flow
[C]	Infra chamber cavity, glass cover slip at base, membrane disc above
[D]	Optical path for upright or inverted long working distance (≥ 5.5mm) optics
[E]	Semi-permeable membrane mounted on acrylic ring supporting slice
[F]	Exit point into supra chamber in vicinity of slice preparation

SCHEMATIC DIAGRAM



A peristaltic pump delivers pulsatile flow at high speed in the range 8 to 15ml / min (A) into a reservoir containing a buffer (B) which induces turbulence and reduces the pulsation. Smoothed laminar flow passes through the infra chamber (C) directly below the surface of the semi-permeable membrane (E) carrying the slice preparation. A Bernoulli effect produces a pressure difference between the upper and lower surfaces resulting in a net movement downwards (arrows) through the membrane and also increases access to slice preparation. A glass cover slip window allows for optical access (D) from below. Fluid exits into the supra chamber (F) and leaves from the chamber through a buffered exit for smooth outflow with a pump or vacuum line.

LOCATION

Most microscope stages will accommodate chambers with an opening up to 4 inches. A plain or holed plate should be available from the microscope manufacturer to fit in the opening, for the MC a minimum hole size of 20mm should be adequate as the opening at the base is 18mm. If there is a larger opening an adapter plate may be required to support the chamber, please contact us for more information. There are four locating holes on the edge of the chamber for adaptation to your microscope stage plate: these are 4mm clearance holes at a radius of 46mm and are also designed to locate on our BSC chamber heating bases with the same fittings for future functions.

CONNECTION OF EXIT WELL

The exit well adjacent to the supra chamber is interconnected by means of a 'U' trap to a second well into which an 'L' shaped steel tube with a slit on the tip is positioned to aspirate excess solution. The function of the 'U' trap is to isolate the slice recording area from mechanical interference caused by the aspiration process. If necessary, the supra chamber exit well can be plugged with nylon mesh of the same type used in the inflow well to help suppress mechanical noise.

The aspiration tube should be connected to a vacuum line with the ability to control the vacuum, this is often done by introducing a 'Y' tube with a gas flow regulator to let air into the line. A peristaltic pump with fast flow may prove more stable and will allow re-circulation of incubating media.

After a few trials it will be possible to set the slice submersion level by adjustment of the vertical position of the aspiration tube. If the chamber is being used for the first time or has been cleaned recently it is sometimes best to leave the chamber filled with aCSF (without glucose added) for a period over night. This helps to establish a hydrophilic surface on the acrylic surface and aids the formation of a negative meniscus which in turn allows for a more stable control of fluid level.

CONNECTION OF THE PERFUSION FLUID SOURCE

Having connected the exit well it should now be possible to connect a source of perfusion fluid to the inlet port [A]. Typically the simplest, cheapest and most stable system is gravity fed from a suitable bottle raised and bubbled constantly with 95% O2, 5% CO2 gas mixture. When a peristaltic pump is used to supply the inflow it is important to use a mesh block in the input well to smooth the flow. The mesh can be cleaned with hydrogen peroxide solution, then rinsed and left in distilled water overnight.

REFERENCE ELECTRODE CONNECTIONS

An earth reference electrode such as a silver/silver chloride pellet may be placed into the EXIT WELL and led out through the plastic fitting normally closed off with a silicone plug. Such a wire may also be pushed further up the inter-connecting tube towards the exit hole close to the insert to give a good ground path.

Noise problems usually arise from external high voltage sources such as mains power cords, computer monitors, oscilloscopes and fluorescent lights. Relocation of these potential sources may be necessary and/or shielding may be required around the recording electrode to avoid these noise problems.

Peristaltic pumps will sometimes also generate very sharp transients due to static discharges along the silicone rubber tubing within the pump mechanism. This may be eliminated by piercing a section of connecting thick walled silicone rubber tubing (at a suitable point close to the chamber) with a piece of chlorided silver wire and grounding this to the central 'spider' ground point of the recording system.

VI
MAINTENANCE

Alcohol should never be used on the slice chamber for cleaning purposes even at low concentrations because it de-hydrates and produces hair-line cracks in acrylic. A special laboratory detergent such as Micro-90 (Registered Name and Trademark of International Products Corp.) which completely rinses out should be used. Heavy deposits of salts should be washed out with distilled water overnight and carbonate salts treated with mild acids such as citric acid. The most common contaminant is fungal growth in the tubes and cavities. This can be avoided by agitated washing i.e. suck out plenty of distilled water intermittently with air bubbles through the tubes and holes of the chamber by use of a powerful vacuum line at the end of each experiment. Continue to dry out by using the vacuum line around all the tubes and also below the removable membrane insert. Leaving the chamber dry will prevent the growth of foreign matter. Cover the chamber with a sheet of clean medical wipes to prevent dust settling on the surfaces. Before the start of each experiment rinse with perfusion fluid.

MAKING A NEW MEMBRANE DISC

A short video for making membrane discs can be found at this link:
http://www.scisys.info/products/graphics/Membrane_Chamber.m4v

DIALYSIS MEMBRANE

Semi permeable dialysis membrane with a 10,000 MWCO is readily available from laboratory consumable suppliers. Please contact us if you have difficulty in obtaining material, some current suppliers are listed in Section VII. A small sample of membrane is included with the chamber as well as a sample ring with membrane attached.

Keep stocks of membrane at room temperature or +4°C in closed original packaging tube to prevent desiccation which can result in cracks or pinholes in the membrane. For best results keep the membrane hydrated by occasionally adding a couple of drops of distilled water to the packaging tube.

REPLACING THE GLASS COVERSLIP

Wear safety goggles for this procedure. Place the chamber upside down on a flat, soft surface (cork tile) and GENTLY remove all the broken glass, using a small flat ended spatula in a scraping action to remove the old silicone rubber sealant. DO NOT use any kind of solvent to remove the sealant. Once completely clear, use a syringe loaded with silicone sealant and bead this around the circumference of the indentation. Place a clean No. 2 thickness, 22mm coverslip on the fresh sealant and GENTLY press ONLY around the circumference of the coverslip. Use a black sealant (Dow RTV732) as it allows the thickness of the sealant to be controlled: darker areas have too much sealant under the coverslip. Ensure the final position of the coverslip is central. Remove excess sealant taking care not to displace the coverslip. Alternatively return the chamber to us and we will replace the coverslip.

VII

SUPPLIERS OF DIALYSIS MEMBRANE FOR MEMBRANE CHAMBER

The dialysis membrane is available directly from regular laboratory suppliers, see below. We currently do not hold stocks or prepare rings with discs as we would not be able to guarantee that they were contamination free.

The details of suppliers are as follows:

UK Thermo Scientific cat. No. 68100 Dialysis tubing SnakeSkin 10kDa Thermo Scientific Pierce Product Code: PN68100 Manufacturer: Thermo Scientific Pierce Manufacturer Code: 68100 Brand: Thermo Scientific Pierce UOM: 10.6m Code: PN	Germany: Fisher Scientific cat. no. 10781755 - SnakeSkin Dialysis Tubing, 10KD, 35 Feet SnakeSkin Dialysis Tubing, 10KD, 35 Feet Artikelnummer: 10781755 Ursprüngliche Artikelnummer: 3168416 Sortiment: Perbio Science / Pierce Marke: PIERCE Bestelleinheit: Stück
Japan: Dialysis tubing SnakeSkin 10kDa Tokyo office Thermo Fisher Scientific Thermo Fisher Scientific office in Tokyo #110-0015 Building 10F 4-24-11 NBF Higashi Ueno, Taito-ku, Tokyo Lab 03-5826-1614 Bioscience 03-5826-1614	USA: Thermo Scientific Thermo Scientific cat. No. 68100 www.thermoscientific.com Contact Phone: +1 800 235 9880 Contact Address: 2650 Crescent Drive Suite #100 Lafayette, CO 80026, United States
China Thermo Scientific Product Code 68100 10K MWCO I.D 22 Contact Phone: +1 800 235 9880 Contact Address: 2650 Crescent Drive Suite #100 Lafayette, CO 80026 United States	Canada Cole-Parmer: http://www.coleparmer.ca **Tel: 800 363 5900** Thermo Scientific SnakeSkin Pleated Dialysis Tubing, 10,000 MWCO, 22 mm dry diameter (ID) x 35 ft RK-02904-10

Additionally Cyanoacrylate adhesive ("Superglue") is not included in the kit.

A brief video here shows the membrane construction process:
https://scisys.info/mc/