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Innovative Engineering for Science

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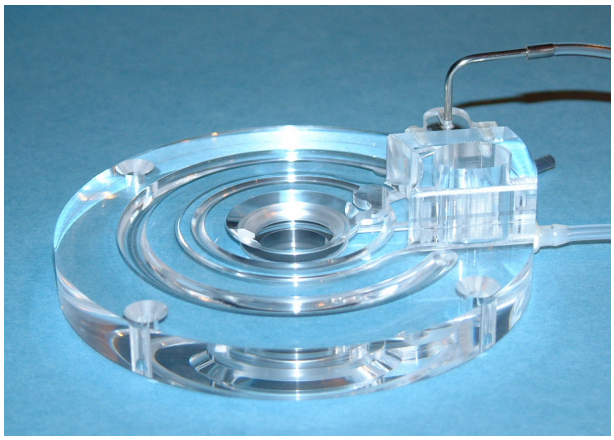
Membrane Chamber

MC

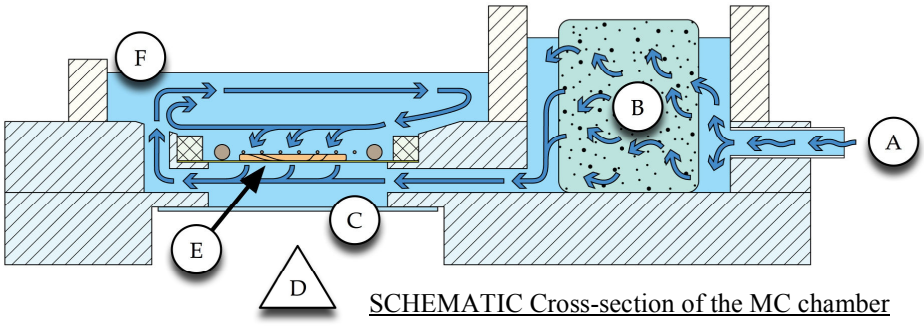
Submerged Preparations



The MC is a new design of *in vitro* slice recording chamber (Hill & Greenfield 2010). Brain slices are submerged on a completely flat, transparent semi-permeable membrane. This offers for the first time ideal conditions for inverted microscopy of *in vitro* preparations. 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 semipermeable 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 coverslip 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.

FEATURES

* *Upright or inverted microscope access to a well perfused, perfectly flat and transparent* in vitro *slice preparation*
* *Slice viability increased beyond 16 hours as demonstrated with LFP recordings*
* *High mechanical stability, high flow rate and Bernoulli effect to optimise nutrient access for slices*
* *Quick replacement of membrane discs and chamber designed for easy cleaning*



SCHEMATIC Cross-section of the MC chamber

A peristaltic pump delivers pulsatile flow at high speed in the range 15ml / min (A) into a reservoir containing a buffer (B) which induces turbulence and reduces pulsation. Smoothed laminar flow passes through the infra chamber (C) directly below the surface of the semipermeable 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 coverslip 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.

References.

Hill, M.R.H. & Greenfield, S.A., 2011 The Membrane Chamber:A new type of in vitro *recording chamber Journal of Neuroscience Methods, 195 (1). pp. 15-23. ISSN 0165-0270*

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FREQUENTLY ASKED QUESTIONS

My perfusion solution forms large globules around the center of the chamber and has difficulty forming a continuous inflow-outflow stream, why does this happen?
This will happen with a new chamber or one that has just been thoroughly cleaned. The polished surfaces of acrylic are hydrophobic, so solutions tend to form globules rather than forming a curved meniscus with the walls and channels of the chamber. To overcome this problem for a new chamber or one that has been thoroughly cleaned, leave a saline (not aCSF) solution to form a pool in the chamber overnight. Rinse as normal before use for the next experiment. We believe that small amounts of salt adhere to the surface thus allowing the meniscus to form.

What is the best method of cleaning the chamber after use?
Rinse with plenty of distilled water and leave dry before the next experiment. NEVER use a solvent of any kind as acrylics tend to fragment and can be completely destroyed. A mild acid (citric) should be used to get rid of deposits, and then left in a large volume of distilled water overnight.

How do I replace the coverslip if it should break?
Wear safety goggles for this procedure. First 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 around the circumference ONLY of the coverslip. It is preferable to use a black sealant as it allows the thickness of the sealant to be controlled: darker areas have too much sealant under the coverslip. Try to ensure the final position of the coverslip is central. Remove excess sealant whilst still wet, taking care not to displace the coverslip. Alternatively return the chamber to us and we will replace the coverslip, please contact us for the cost of this service and further details.

Where is the best electrical ground point?
This will have to be found by trial and error. In addition to the AgCl type wire electrode you can ground the inlet and outlet tube by inserting a section of stainless steel hypodermic tubing and grounding to help to eliminate any artefacts from the suction line. Quite often it helps to push a grounding wire INTO the wall of silicone rubber tubes that you use for connection to your perfusion solutions, either or both the input and exit lines.