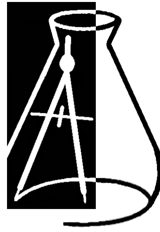

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Innovative Engineering for Science

BRAIN SLICE CHAMBER SYSTEM

BSC1

Scientific Systems Design Inc. 1997.

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 UNDER ANY CIRCUMSTANCES OPERATE THE PTC03 TEMPERATURE CONTROLLER AND BRAIN SLICE CHAMBER WITHOUT ADEQUATE WATER IN THE LOWER CHAMBER OR WITH THE SENSOR PROBE REMOVED FROM THE CHAMBER END. THIS CAN CAUSE OVER-HEATING OF THE HEATER ELEMENT. A THERMAL FUSE IS LOCATED IN THE SLICE CHAMBER TO PREVENT WATER TEMPERATURE RISING ABOVE 70°C. DO NOT LEAVE THE CHAMBER RUNNING UNATTENTED FOR EXTENDED PERIODS OF TIME - PLEASE CONTACT US FIRST FOR DETAILS IF YOUR EXPERIMENTS NEED TO RUN OVERNIGHT AND UNATTENDED.

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.

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I

CHAMBER DESCRIPTION

The BSC1 Brain Slice Chamber was designed to maintain isolated, living tissues *in vitro* and allow stable electrophysiological recordings to be made from the preparation. Both 'interface' and 'submerged' methods of maintaining slices can be achieved with the same chamber without any additions. Temperature is maintained by a proportional control heating system.

CONSTRUCTION

The chamber is constructed from two cylindrical blocks of acrylic, and mounted on a base-plate of the same material for securing on to a table. The diameter is 100mm, height is 85mm, base plate diameter is 130mm. The lower section maintains the desired temperature and provides a moistened 95% oxygen, 5% carbon dioxide gas mixture to the upper section. Here the slices rest on a nylon net fixed on to a removable insert, located in the centre of the chamber. The gas mixture is deflected downwards and across the surface of the slices located centrally.

The length of the inlet channel within the upper chamber has been kept as short as possible to minimise the fluid 'dead space', so as to allow rapid changes of solutions which are required in pharmacological experiments. Pre-oxygenated perfusion fluid enters the main body through a fine bore tube which spirals in the heated distilled water contained in the lower chamber and finally enters the upper part via a bubble-trap.

II

METHODS OF PERFUSION

The perfusion fluid can enter by two different methods depending on the way in which the slices are to be maintained, either as an 'INTERFACE' or as a 'SUBMERGED' preparation. Interface preparations are normally used for exploration of field potentials since they offer good visualisation of laminated regions such as cell body layers which appear relatively translucent and fluid bathes only one side. Submerged preparations offer fluid exchanges over both slice surfaces when experimenting with ionic concentrations and perfusion of drugs. However, dead space has to be taken into consideration as the test solution will take longer to rinse out.

INTERFACE

In this type of preparation the height of the perfusion fluid is adjusted so that it is virtually at the same level as the surface of the slice resting on the nylon net. In this way the solution forms a very thin film over the slice keeping it moist. This type of interface preparation requires a high oxygen tension in high humidity maintained above the slice. With Krebs solutions, this is achieved by passing a 95% oxygen, 5% carbon dioxide gas mixture through a bubbler located in the lower part of the chamber containing heated distilled water. The pre-oxygenated incoming perfusion fluid enters the chamber via a tube from the side where it first spirals in the heated distilled water contained in the lower part. This tube then enters the upper part of the chamber through a bubble trap, ensuring that any gas that has come out of solution in the form of small

bubbles does not get trapped under the slice and disturb the preparation during electrophysiological recordings. This excess gas is released to atmospheric pressure or optionally removed from the trap by means of a syringe attached via a tube. After passing the bubble trap the solution reaches directly under the nylon net on which the slice is supported, usually resting on a piece of lens tissue. The solution then exits through a hole located at one side, which leads into a well where it is removed by means of a hypodermic needle attached to a suction line. The height of the needle is adjusted by turning the acrylic knob on top of the chamber, this allows the fluid level to be set to the desired level and maintained constant, usually at the level of the surface of the slice. With a 0.5mm fluid depth above the insert net, the total fluid dead space is approx. 3 mls. Approx. 0.5 ml of this volume is in the inlet PTFE tubing within the chamber.

SUBMERGED

In this type of preparation the solution height is adjusted to be above the surface of the slice, by approximately 0.5mm. The perfusion fluid enters below the nylon net as before and circulates around the slice before exiting through the well.

III

OXYGENATION

INTERFACE

The oxygen tension above the slice is maintained by an inflow of a pre-humidified 95% oxygen, 5% carbon dioxide gas mixture from the warmed lower part of the recording chamber. Although the warmed distilled water in the bottom of the chamber will provide much of the humidity in the upper chamber, it is very important to have the gas mixture pre-humidified before it enters the chamber. This is usually done with a "gas wash bottle" fitted with a sintered glass bubbler. The gas enters the upper section along a concentric channel and is then deflected down by the lid towards the centrally located preparation

SUBMERGED

A high perfusion fluid flow rate (up to 20ml/min) is usually employed with this type of preparation, the pre-oxygenated solution around the slice normally carries all the required oxygen. The flow of oxygen above the slice is therefore not as critical and for this reason it is sometimes permissible to remove the lid altogether to give greater access for positioning the recording/stimulating electrodes. The conical profile leading to the slice allows electrodes to enter at a 45° angle, to permit good visualisation from above with a dissecting microscope.

IV

TEMPERATURE

PROPORTIONAL TEMPERATURE CONTROLLER PTC03

DESCRIPTION

The PTC03 is a temperature control unit for use with the slice chamber. A low voltage direct current output with low noise characteristics is used to power the heating element contained within the lower chamber together with a sensor for feedback proportional control. The required temperature is set using the front panel control with a digital read-out of set temperature. When the display selector is set to control the display reads the temperature of the control sensor. Provision is also made to display the temperature from an optional monitor sensor if this is being used. Full control is reached within 20 minutes at a setting of 40°C, with an ambient of 20°C. Set temperature must exceed ambient by 2 ^{o}C minimum.

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

INTERFACE

The upper chamber temperature is maintained by ensuring that the gas mixture and the physiological solution enter at the required temperature, this is dependent on the temperature of the body of the chamber which is warmed by a heating element controlled by the PTC03. The whole system reaches equilibrium within 20 minutes.

SUBMERGED

The higher flow rate used with this method allows a more rapid settling of the final temperature. Again with this method the inflow of pre-warmed gas mixture can be reduced as it will only have a minor contribution to the final temperature, however it is necessary to maintain gassing in the lower chamber as this provides a stirring action enabling efficient heat transfer between the heater and sensor probe of the PTC03.

ALTERNATIVE METHODS OF TEMPERATURE CONTROL

Both the heating element and temperature sensor probe can be unscrewed to allow an alternative method of heating to be used with the chamber. Adaptors are available to replace the two hole positions with a heat exchanger which can be fed from a circulating hot/cold water bath. Please ask for further details.

INSTALLATION AND OPERATION

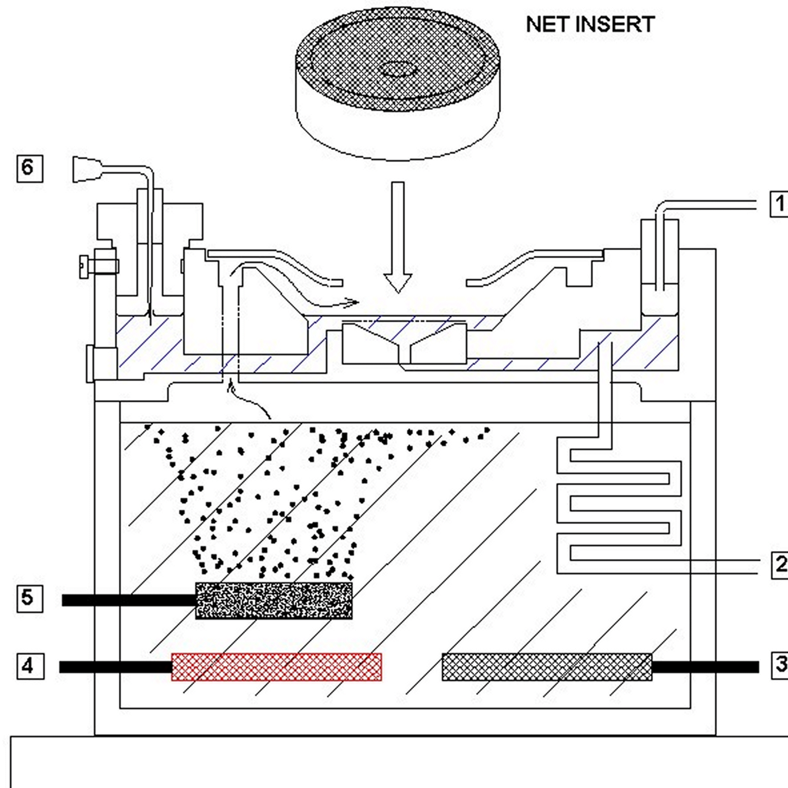
Your parcel should contain both the Brain Slice Chamber and the PTC03 Temperature Controller. Once all packing material has been removed, PLEASE take some time to examine the construction of the chamber. Remove and identify: one removable insert with nylon net, one lid, a pack of small plastic strips for locating the net insert.

All the connecting tubes and wire ports [1] to [6] are LABELLED ON YOUR CHAMBER as follows:

LABEL	FUNCTION
[1]	Bubble trap for perfusion fluid entering through PORT (2)
[2]	PORT for incoming perfusion fluid
[3]	Probe SENSOR for temperature control (plug end connected to PTC03 SENSOR socket)
[4]	Heating element connection. The connecting cable is fitted with a blue three pin plug for connection to the PTC03 HEATER socket.
[5]	Gas bubbler for incoming oxygen/carbon dioxide gas mixture.
[6]	Exit well for waste perfusion fluid removed via hypodermic needle which should be connected to a vacuum line via a waste bottle.

Do not attempt to dismantle the chamber at this stage, it should rarely be necessary to do so.

SCHEMATIC DIAGRAM



LOCATION

The chamber should be secured to a solid, smooth table surface such as marble or steel. (Steel should either be coated with an anti-corrosive film or nickel electro-plated). The four 90 degree pattern fixing holes in the base can be used to mount small magnetic bases if a steel table is used as a base. The base-plate must not become distorted through securing on to an uneven base, nor should nuts and bolts be over-tightened in the holes provided.

FILLING LOWER CHAMBER

Once secured, fill the lower chamber with approx 110 mls of distilled water using a syringe fitted with a plastic tube capable of being inserted into one of the eight vent holes on the upper section. Make a note of the fill level which should be seen to completely immerse the heating element visible in the lower chamber and be 2 to 4 mm below the junction between the upper and lower sections. Check this level routinely on a daily basis before switching on the power to the system. Once a week at the end of the day switch off the power and use a fast vacuum line to suck out as much as possible the distilled water in the lower section, rinse and refill with fresh distilled water as before to the correct level before switching on power. This operation prevents the growth of foreign matter.

CONNECTION TO GAS MIXTURE SOURCE

The gas mixture source should in addition to its reduction valve have a secondary flow regulator for fine adjustments. Once connected to PORT LABEL [5] adjust according to whether interface or submerged mode is being utilised. In addition to providing warmed and moistened gas to the upper chamber, this gassing is necessary to keep the lower section distilled water stirred for efficient feed-back from the heater to the sensor connected to the PTC03 Temperature Controller.

CONNECTION TO TEMPERATURE CONTROLLER

Check that the SENSOR probe is inserted into its hole (LABEL [3]) and that the plug end is connected to the PTC03 Temperature Controller SENSOR socket. Connect the heater power cable from the chamber to the HEATER socket on the PTC03 Temperature Controller. Connect the mains power lead to a suitable socket WHICH MUST HAVE AN EARTH CONNECTION for safety and low noise operation. Turn on the power switch located on the rear of the PTC03. On the front panel the 'LINE ON' light should now be on. Move the selector switch to 'SET', a light above the temperature adjustment knob will turn on to indicate 'SET' mode. Adjust the knob and read the LCD display to set to a desired temperature in ^{o}C. Once set move the selector switch to 'CONTROL'. Assuming you have selected a temperature above ambient, the 'HEATER ON' light will glow brightly or dimly depending on how close the lower chamber temperature is to the set temperature.

NOTE. The temperature shown on the LCD display will be the temperature of the lower chamber distilled water. The temperature achieved in the upper chamber at the location

of the preparation depends on a number of factors principally:-

1) Whether the preparation is in ‘interface’ or ‘submerged’ mode
2) Ambient temperature
3) Incoming gas mixture flow rate
4) Perfusion fluid flow rate and initial temperature (e.g. from the refrigerator?)
5) Whether chamber lid is in position

Since the above factors are quite stable during the course of an experiment, there is a fixed temperature differential between the upper and lower sections of 3 to 4 degrees for interface and submerged modes. Given this differential, the PTC03 effectively controls the upper chamber temperature which should be monitored with an independent miniature (eg. thermo-couple type) temperature probe. Allow at least 10 to 15 minutes for the system to equilibrate, and approximately 5 minutes for a 5°C temperature increase but 20 to 30 minutes for a 5°C temperature decrease.

As part of our program of continual improvements, provision is already made on your PTC03 circuitry for a plug-in monitor temperature sensor (select MONITOR on switch). This sensor should be available in the future when a more compact version is designed and capable of insertion close to the slice preparation.

<u>CONNECTION OF EXIT WELL</u>

Submerged and interface modes will both require operation of the exit well LABEL [6] from which the fluid height is set. By means of a hypodermic needle connected to a vacuum line, it is possible to achieve control of the fluid height above, or at the level of the slice preparation. A nylon screw and rotating acrylic top allows the needle to move up or down. To make this adjustment, slightly loosen the small screw on the side of the exit well which secures the acrylic top. Whilst holding the nylon screw assembly steady, turn the acrylic top and observe clock-wise turning of the top raises and anti-clock turning lowers the assembly. Re-tighten the small screw on the side to avoid accidental alteration of fluid level during an experiment. The vacuum line should be connected via a waste bottle to smooth out any irregularity. In the absence of a house vacuum point, typically a high pressure water vacuum adapter is used, electric pumps are equally effective. A bleed valve is recommended when utilising powerful electric pumps to allow adjustments of the level of vacuum, excessive or inadequate levels will cause problems. Peristaltic pumps are not recommended for this purpose as the high flow rates are not readily achieved and unless an expensive version is available, the flow will not be smooth resulting in an unstable fluid level. The correct vacuum level will be found by trial and error, depending on perfusion flow rates. Try pouring a few mls of perfusion fluid into the centre of the upper chamber to see how the fluid behaves with your selected vacuum line. A good sign of stability once the perfusion system is connected up is a constant ‘hiss’ from the exit well. Use an ultrasonicator to clean the needle once weekly, or unclip and remove the old one and replace with a new one of the same diameter bent to the same shape and re-fit into the nylon screw.

NOTE.
1)When the chamber is new the polished acrylic surfaces tend to be hydrophobic, so fluids form a ’positive’ meniscus on surfaces which give rise to instability in fluid control. For this reason it is recommended that the chamber slice area is submerged in aCSF (without glucose) over-night for one to two days. This ‘conditions’ the acrylic plastic to become more hydrophilic and fluid level control stabilises.

2) If problems are experienced in achieving constant fluid height due to poor vacuum lines, remove the acrylic top and nylon screw assembly by loosening the small side screw and try a larger bore hypodermic needle (in the case of a high vacuum source) mounted on a simple micro-manipulator close to the side of the chamber and lower the needle into the exit well to achieve control.

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 LABEL[2]. Typically the simplest, cheapest and most stable system is gravity fed such as a raised blood-drip set filled with the desired perfusion fluid, pre-gassed with 95% oxygen / 5% carbon dioxide gas mixture or a suitable bottle raised and bubbled constantly with the above gas mixture. A blood-drip set has the advantage of allowing the flow rate to be monitored from the drip rate, in addition the flow adjustment clip is usually easy to operate.

BUBBLE FORMATION

In order to prevent excessive bubble formation around the slice preparation, the incoming aCSF solution should be pre-saturated with oxygen close to or at the same temperature as the brain slice chamber. This is because oxygen solubility in water at 5° C is almost twice that at 37° C, therefore oxygenating aCSF retrieved from the refrigerator and perfusion of this to the warmed slice chamber will result the in release of oxygen bubbles out of the solution. When the incoming perfusion solution is pre-warmed and saturated in this way, a small number of bubbles may still appear in the solution, and many will be captured in the bubble trap. The tube entering through PORT [2] spirals in the lower chamber before it passes from underneath to the bubble trap LABEL [1]. The top end of the bubble trap may be either left open to atmospheric pressure (remove screwed fitting and associated tube) or connected by the connecting tube provided to a small glass syringe (closed system). The latter system is necessary if high perfusion rates are adopted, the occasional bubbles collected in the trap are then removed by use of the syringe.

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 either one of the two side vents found immediately below the level adjustment device. 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. If excessive electrical noise problems arise, arrange for a piece of chlorided silver wire in the form of a tight spiral approx. 3 mm in overall diameter to be positioned by use of a micro-manipulator close to the recording site on the nylon net of the insert.

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.

The heating element in the chamber is driven by a low voltage, low noise direct current power source. If it is found that on switching off the power to the PTC03 (whilst the mains plug is still in the power socket) that noise is eliminated, check the earth

connection at the mains plug and socket.

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 silicone rubber tubing (at a suitable point close to the chamber) with a piece of chlorided silver wire and grounding this to the central grounding point of the recording apparatus.

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 laboratory detergent 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 upper section 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 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.

REPLACING NYLON NET ON INSERT

1) Clean the rim of the old insert by scraping off the worn net, making sure all residual adhesive is removed. Use a laboratory detergent for final cleaning, wash in distilled water and leave to dry.
2)Apply a coat of cyanoacrylate based adhesive "super-glue" on to the surface of the rim.
3)Stretch a piece of suitable netting over a rigid circular tube (eg. 40mm diameter container) kept in place with rubber band. Pull net taut by pulling around rubber band.
4)Invert the insert with the adhesive coating and place over the stretched net making sure no contact is made with rim of the circular tube. Leave the insert to stand under its own weight for 24 hours.
5)Cut the rubber band to release the net and insert intact. Cut the net around insert using curved scissors if possible. Wash again in distilled water before final use.

Locate replacement insert in chamber after cleaning all deposits around insert well - do not use any kind of solvent as this will damage the acrylic chamber. A mild acid such as citric or acetic will help removal of deposits, hydrogen peroxide will also assist removal of fungal growths. Thoroughly rinse with distilled water afterwards or leave a pool of distilled water in the chamber well overnight. Inserts are also made of acrylic and therefore must be treated with the same care.

Locate stainless steel fixing screw into center hole of plastic retaining strap and screw down firmly so that one end of the strap rests on the sloped chamber wall and the other rests on the insert to clamp in down firmly.

Remove and clean insert and surrounding areas as described above at least once per week. At the end of each experiment, flush through the system with an agitated stream of distilled water or suck through the tubes with a powerful vacuum line ensuring agitation by allowing air to intermix with the stream of distilled water. The bubbles will assist in removal of growth lodged in the tubes of the chamber.