

Technique for setting up the perfusion of a recording chamber for physiological studies with cultured and acutely dissociated cells

Trese Leinders-Zufall

Department of Anatomy and Neurobiology and the Program in Neuroscience

University of Maryland School of Medicine, Baltimore, Maryland

1998

In many electrophysiological and dynamic imaging experiments, controlling the speed and height of the perfusion solution in the recording chamber is often not achieved in a satisfactory manner. A method (Fig. 1) is described here for controlling the flow of the recording solutions and therefore the precise height of the solution in most recording chambers of Warner Instrument Corporation. This report is an extract of a chapter titled 'Regulating the perfusion speed and height of microscope-mounted chambers' which will be published in 'Electrophysiological Methods for the Study of the Mammalian Nervous System' (Kocsis JD (ed), Appleton & Lange).

Control of the recording chamber input

The flow of the perfusion solution into the recording chamber can be controlled by using either a pump or by gravity feed. In many laboratories it seems that gravity is one of the most commonly applied methods. However, in using this method, the speed of the solution has to be strictly controlled. There are various ways to achieve that. Some use various tubing length and diameter combined with securing the bottle or syringe containing the bulk of the perfusion solution on a specific height. This is the cheapest way, but

not the most precise one. Other methods use clamping devices such as screw compressor clamps (Fig. 2a) or tubing clamps (Fig. 2b) to control the flow rate. Often this method is used in combination with a dripping balloon (Fig. 2c) to visually observe the speed of the perfusate. Instead of using the various clamping devices, a more accurate method involves the use of gravity flow controllers (Fig. 2d) that are originally designed to control the flow of intravenous solutions for hospital patients.

An often overlooked factor for the precise control of the solution flow into the recording chamber is the container that holds the bulk of the perfusion solution. When using a syringe, 'separatory funnel with a stopcock' or any other device with gravity pressure, the speed of the solution will vary

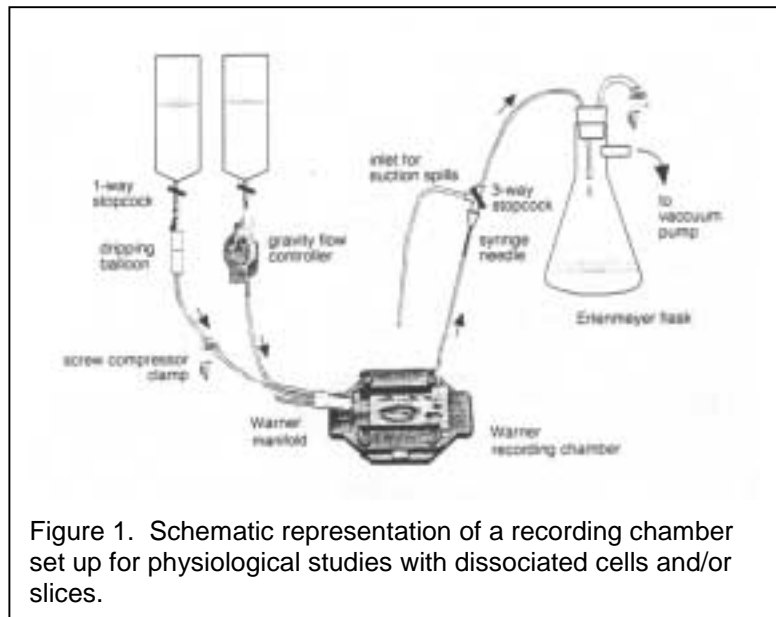


Figure 1. Schematic representation of a recording chamber set up for physiological studies with dissociated cells and/or slices.

proportionally to the amount of perfusate left in the container. This is caused by the 'weight loss' of the solution left in the container that provides pressure onto the flow controller. There are two ways to circumvent this problem. One might use e.g. a very large perfusate reservoir. The amount which is then lost from the container to the recording chamber is then relatively small in comparison to the remaining volume. However, it is common to forget to keep the amount of the perfusion solution above a specific point for maintaining a similar pressure on the tubing lines. For that reason, Nigel Cox of Warner Instruments devised another solution which makes use of 60 cc glass syringes. These syringes are easy to clean and can even be sterilized or decontaminated when necessary. The glass syringes are equipped with a hollow glass plunger. If this plunger is cut open at the top and filled with weights (e.g. sand) and placed on top of the perfusate in the syringe, this weight will cause a constant pressure on the flow controllers.

In addition to controlling the flow rate of the solution, it is a good idea to have a 1-way stopcock directly at the source of the perfusate. This enables instantaneous stoppage of the solution flow, which is advantageous if more than one perfusate will be used during the experiment. Instead of the stopcock some investigators use roller clamps. However, these devices clamp down the tubing in such a way that they cause over time blockage of the solution flow due to the permanent dislocation of the tubing.

In the event that more than one bath perfusate is being used, Warner Instrument Corporation has manifolds available for up to 8 input lines. The inputs are joined internally to a common output in a near zero dead space configuration. These manifolds should be used with a very short length tubing between the manifold and the recording chamber, so the solution exchange times are minimized. In combination with the manifolds, the already mentioned 1-way stopcocks (see Fig. 1) should always be present to prevent the back-propagation of the flowing perfusion solution into the tubing of the other perfusion solutions.

Control of the recording chamber output

The perfusion solution in the recording chamber can be removed by using vacuum suction, in the event that not a pump is operating the in- and output. When using vacuum suction, it is necessary to control the force of the suction precisely to be able to fine tune its height in the recording chamber. This can be achieved by using the appropriate tubing, a stopcock and the metal suction device delivered with the recording chamber.

The metal suction device is specially designed to draw air and fluid at the same time, which helps to control the solution height more accurately and also reduces electrical noise caused by the suction vortex. The ideal position of this metal suction device is such that the longitudinal opening is kept in a 90 degrees angle to the solution surface. The suction device can be easily placed at any location in the recording chamber reservoir with the use of modeling clay, in the event that the designated holder built into the chamber is at an inconvenient position.

Polyethylene tubing (PE-160, 1.14 mm ID, 1.57 mm OD) is then connected to the metal suction device by using tubing that fits snugly both the PE-160 tubing and the suction device. This PE-160 tubing ends onto a stopcock. So, that when stopping the solution flow at the input level, the suction can also be shut down to prevent drying out the recording chamber. An easy way to connect the stopcock to the PE-160 tubing is to use a 18G1 syringe needle which has the sharp tip removed. In principle only a 1-way stopcock is needed, but it is convenient to use a 3-way stopcock. The extra inlet can then be used for suctioning spills or for quick cleaning of the recording chamber.

The other side of the 1-way or 3-way stopcock will be connected to an Erlenmeyer flask that is used for collecting the waste solution (see Fig. 1). The tubing size on this side is irrelevant and usually a larger size than the PE-160. The Erlenmeyer flask has two inlet openings to regulate the force of the vacuum suction. The second inlet is

connected to tubing fitted with a screw compressor clamp. By changing the diameter of the tubing, the suction force is changed on the outlet line of the recording chamber. The force of the suction is now adjusted to the inlet flow speed. The fluid level in the recording chamber can then be easily adjusted by raising or lowering the metal suction device in the recording chamber reservoir.

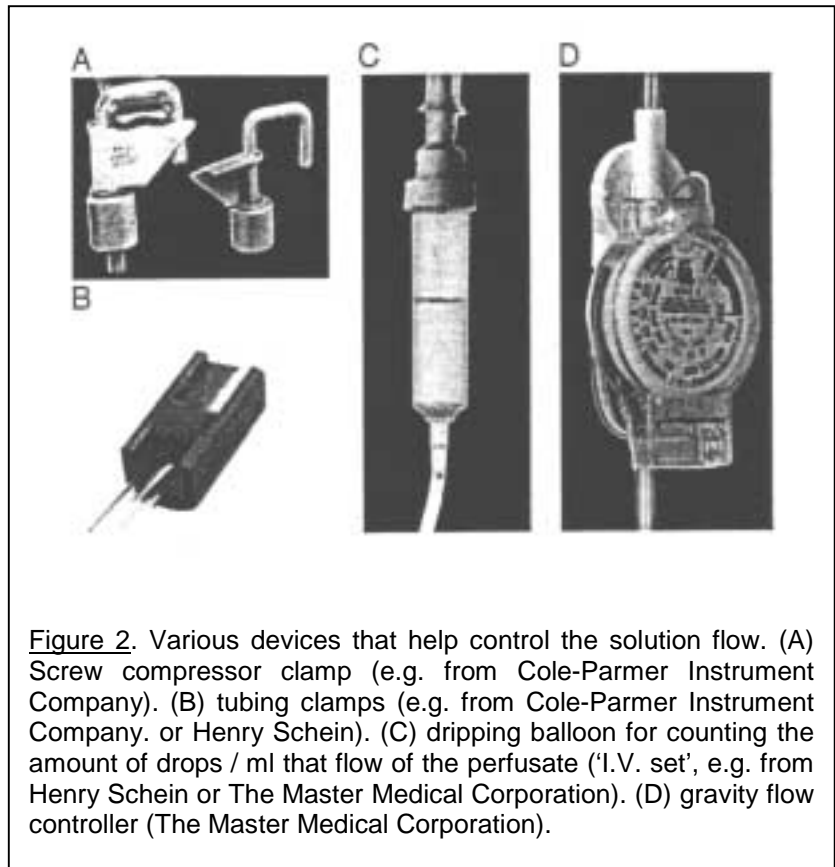


Figure 2. Various devices that help control the solution flow. (A) Screw compressor clamp (e.g. from Cole-Parmer Instrument Company). (B) tubing clamps (e.g. from Cole-Parmer Instrument Company. or Henry Schein). (C) dripping balloon for counting the amount of drops / ml that flow of the perfusate ('I.V. set', e.g. from Henry Schein or The Master Medical Corporation). (D) gravity flow controller (The Master Medical Corporation).

Acknowledgment

I thank Drs. Adam Puche, Shan Chen and Roland Bock for comments on this document.