Technical Note

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Title: **Millicell Organotypic Cell Culture Procedure**

NOTE: This is an example of a typical organotypic culture protocol. Other methods, including ones with no pre-coating, have been applied with equal success.

Millicell cell culture plate inserts are widely used in cell and tissue culturing applications. The Millicell-CM, with Biopore membrane, is particularly well suited to organotypic cell cultures. This unique membrane is extremely biocompatible. Explants readily adhere to its surface without the addition of an extracellular matrix coating. Additionally, the Millicell-CM culture plate insert comes in two diameters 12 mm and 30 mm and can be easily placed into standard 6- or 24-well plates for culture.

For those researchers who wish to analyze their excised tissue through microscopy, Millipore provides a new low-height version of the standard Millicell. The new Organotypic Millicell (Millipore catalogue number PICM 0RG 50) is a 30 mm device with a very low profile: only 5 mm overall height. This new shorter version greatly facilitates ease of handling, so samples can be readily accessed with a microscope or electrophysiology equipment without modifying the Millicell or removing the membrane from the supporting ring. Additional information regarding Organotypic Cell Culture, Millicell Culture Plate Inserts and other cell culture applications and accessories is available from your local Millipore sales representative or at our website at www.millipore.com/millicell.

Stationary Organotypic Cell Culture is a method for growing and maintaining healthy tissue slices without the use of rocker plates or roller tubes. In this type of cell culture, explants of healthy tissue are cultured on the Millicell CM inserts at the interface between culture medium and a CO_2 rich environment. The following protocol is based upon the pioneering work of Dr. Luc. Stoppini of the Department of Pharmacology at the University Medical Center in Geneva, Switzerland, and is intended as a basic guideline for those researchers interested in hippocampal explant culturing or organotypic cell culture in general. It may be necessary to modify reaction times and reagents used based upon the tissue types selected for study.

MATERIALS NEEDED (Culturing and Microscopy)

- Millicell-CM (PICM0RG50) - no ECM required; pretreat with 10 µl of 1 mg/ml polyornithine solution
- Thinly dissected tissue explant (e.g., hippocampal slices)
- Culture Medium:
  50% MEM (Gibco 079-01.012)
  25% Horse Serum
  25% HANKS solution (buffered by addition of 5mM TRIS and 4mM $NaHC0_3$ pH 7.2)

Penicillin and streptomycin may be added.
Glucose may also be added to render concentration of 6.5mg/ml in each solution made.

- Rinse Solution: 0.1M phosphate buffer pH 7.3
- Fixative: 1.5% glutaraldehyde and 1.0% paraformaldehyde in 0.1M phosphate buffer (pH 7.3)
- Primary Dehydration Solution: 25, 50, 75, 95, and 100% acetone solutions
- Secondary Dehydration Solution: PO (propylene oxide)
- Infiltration Solution: 1:1, 1:3 solutions of PO:EPON
- Embedding Solution: 100% EPON
- Light Microscopy Stain: methylene blue and azur II in borax
- Electron Microscopy Stain: aqueous uranyl acetate and lead Citrate
- Polyester foils
- Sterivex-GS

PROCEDURE

Culturing

1. Excise the area of interest from the tissue sample and store in medium (see formulation above) until all tissue is harvested.

Please note: All media should be prefiltered using the Sterivex-GS

2. Pretreat the Millicell-CM (PICM0RG50) with 10µl of a 1 mg/ ml solution of polyornithine.

3. Place the explants (using a cut Pasteur pipette) on the membrane, being certain to maintain a drop of original culture medium in the pipette with the explant. This small droplet will help form the minute film of media that must be maintained on the surface of the explant to keep it humid. In general, only enough medium should be added to the explant to cover the tissue slice with a thin film of liquid. Tissues must well exposed to the air (CO_2 enriched) to promote healthy development and maintenance of tissue slices for long term culture. Additionally, several slices may be placed on a single membrane. The Millicell-CM then may be placed in a petri dish containing medium. In general, for a 35 ml petrie dish, 1.1 ml of media is used.

Microscopy

Dehydration and fixation

1. Rinse slices in 0.1M phosphate buffer pH 7.3.

2. Fix slices in 1.5% glutaraldehyde and 1.0% paraformaldehyde in 0.1M phosphate buffer (pH 7.3) for 2H at 4°C.

3. Slice away membrane around culture*.

4. Rinse explant in phosphate buffer (pH 7.3, cold solution) for 1H at 20°C in the dark.

5. Rinse 3 more times for 15 mins each in phosphate buffer.

6. Dehydrated samples through an ascending series of acetone concentrations (25, 50, 75, 95%) for 10 mins., each followed by 3 changes of 100% acetone for 20 minutes each.

7. Replace acetone with propylene-oxide (PO, 2 changes of 5 minutes each).

8. Infiltrate samples through graded PO:EPON mixtures (1:1, 1:3; 2H each).

9. Store overnight in 100% EPON.

Embedding

1. Embed slices flat in EPON between transparent foils for 48 Hr. at 600°C.

2. Remove olyester foils and the thin blocks of tissue re-embedded in EPON.

3. Polymerize at 60°C for 2 more days.

Staining

1. 1-2 µm thin sections can be stained in a solution of methylene blue and azur II in borax for light microscopy.

2. Ultra thin sections of 60-80 nm can be mounted on uncoated copper grids and stained with aqueous uranyl acetate and lead citrate.

3. Section can be examined with Philips EM300 electron Microscope at 80kV (or equivalent).

References:

1. Stoppini, L., Buchs, P. A., Muller, D.," A simple method for organotypic cultures of nervous tissue" **Journal of Neuroscience Methods**, Vol.37, pp.173-182, 1991.

2. Buchs, P. A., Stoppini, L., Muller, D., "Structural modifications associated with synaptic development in area CA! Of rat hippocampal organotypic cultures" **Developmental Brain Research**, 00 (1992)BRD51565.

3. Belenky, M., Wagner, S., Yarom, Y., Matzner, S., Cohen, S. and Castel, M.,"The suprachiasmatic nucleusin stationary organotypic culture" **Neuroscience**, Vol. 70, pp.127-143, 1996.

Filter Brand Name	Filter Code	Filter Material	Filter Pore Size, µm	Device Diameter Outer at Top, mm	Catalog Number / Order
Organotypic culture applications, reduced height facilitates viewing, inert and transparent, ECM required					
Biopore	CM	PTFE	0.4	30	PICM0RG50 add to order