Preparation of Costar® Transwell® Inserts for Scanning Electron Microscopy

Supplies

Reagents
Sodium cacodylate (Polysciences Cat. # 01131)
3% Glutaraldehyd E.M. grade (Polysciences)
Osmium tetroxide - crystalline (Polysciences Cat. # 0233A)
Sucrose, reagent grade
Ethanol, 95% and 100%
Hexamethyldisilazane (HMDS) (Polysciences Cat. # 00692)
Colloidal Silver Paste (Polysciences Cat. # 03208)
Hank's Balanced Salt Solution (HBSS)

Materials
Double-sided sticky tape

Protocol

1. Rinse the Transwell membrane with cells by adding HBSS (37°C) to the bottom of the plate well until it reaches the membrane, and then add HBSS to the insert side of the membrane. The rinse should be added slowly down the insert side and then aspirated. Do not touch either surface of the membrane. Repeat the rinse procedure.

WARNING: Appropriate safety precautions must be used when handling fixative solutions and the highly reactive osmium tetroxide, which gives off a reactive vapor.

2. Replace the HBSS with a primary fixative (3% glutaraldehyde in 0.1M sodium cacodylate, pH 7.4, containing 0.1M sucrose) to adjust the osmolarity to approximately 300mOsm. The fixative is added to both the well and the Transwell insert for 45 minutes at room temperature. **This procedure should be conducted in a fume hood.**

3. Remove the primary fixative and add the cacodylate-sucrose buffer (0.1 M sodium cacodylate, 0.1 M sucrose, pH 7.4) for 5 minutes at room temperature. Remove the buffer and repeat this rinse procedure for an additional 5 minutes.

4. Replace cacodylate buffer with the secondary fixative (1% osmium tetroxide in the cacodylate-sucrose buffer, pH 7.4). Fixation should proceed for 1 hour at 4°C. **Appropriate safety precautions must be used when handling the highly reactive osmium tetroxide as it gives off a reactive vapor. Use latex gloves and have a proper disposal bottle available for the used osmium.**

5. Remove the osmium tetroxide and rinse twice (5 minutes per rinse) as above. The rinses should be collected in the osmium disposal bottle. At this point, the samples may be refrigerated in the cacodylate-sucrose buffers. If prolonged storage is necessary, rinse the cultures with fresh cacodylate-sucrose buffer after 48 hours.

6. Remove the cacodylate-sucrose buffer and dehydrate the insert by gently adding solutions of ethanol in a graded series of concentration as follows:
   35% Ethanol -10 minutes
   50% Ethanol -10 minutes
   70% Ethanol -10 minutes
   95% Ethanol -10 minutes
   100% Ethanol -10 minutes
   100% Ethanol -10 minutes

   Note: Do not let the membrane dry out at any time during the procedure.

Drying

1. The insert may now be dried in a critical point drier using ethanol (not acetone) and liquid CO_2 according to the instrument instructions. A more convenient and economical technique that will produce excellent results is the use of hexamethyldisilazane (HMDS**). HMDS must be used in a fume hood.**

   a) Replace the last 100% ethanol solution with HMDS or quickly transfer the Transwell insert to a container with enough HMDS to cover the insert.

   b) Leave the insert in HMDS for 10 minutes.

   c) Air-dry in the hood for 20 to 30 minutes. **Note:** Store in a desiccator if the membrane is not to be mounted immediately.

Mounting

1. The membrane may be mounted in either of two ways:

a) The membrane can be removed from the Transwell insert with a clean, small scalpel blade (#11 or #15). Be careful not to touch the surface of the membrane and avoid unnecessary bending. Small membranes may be mounted whole, and larger membranes may have to be carefully cut into small pieces with the scalpel blade or sharp scissors. Place a piece of double-sided sticky tape on the top of an aluminum stub and place the membrane on the tape.

b) The major portion of the insert holder can be cut away with a hot scalpel blade and the plastic ring holding the membrane can be attached to the aluminum stub with Silver Paste. The paste must be allowed to dry thoroughly in a desiccator. **Note: If the samples are not to be coated immediately, they must be stored in a desiccator.**

2. Sputter coat the samples with 200 Angstroms of Gold-Palladium and examine.

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