A Sticky TEM Grid

Very often, we wish we could have a sticky copper grid to hold our TEM samples. Here is a small trick to create sticky grids.

1) Mix a drop of 5 minutes epoxy in a petri dish
2) Dilute this epoxy in Acetone in about a 20:1 ratio
3) Immerse the copper grid you want to use into this solution
4) Remove the grid and blow air on the grid from the side to remove excess epoxy and to open up the grid openings.

That's it. Now you have a TEM grid covered with a very thin layer of 5 minutes epoxy only on grid bars.

I use this sticky grid for semiconductor TEM sample preparation to hold the epitaxial layer when the substrate is chemically removed. A sticky grid is laid down on the area of interest. Wait 5 minutes for epoxy to cure. Then I can start my selective etching process from the backside of the sample.

After etching, the thin and fragile layer of semiconductor (less than 1 μm) will be held by the grid to give enough mechanical strength for handling. You can certainly think of other applications.

Hel-Ruey Harry Jen, AMP Inc.

TEM of Cultured Cells on Coverslips

For TEM of cultured cells, we grow the cultures on "Thermanox" tissue culture coverslips (from Nalge Nunc International, 50 sterile coverslips, 13 mm diameter is catalog 174950). The coverslips can be treated with all the same chemistry as tissue, including propylene oxide and Spurr's epoxy, which are two components which solubleize polystyrene.

Coverslips are easily processed in disposable propopylene 50 mL centrifuge tubes, useful since the cell culture will not contact the wall of these conical tubes.

1) Sink the Thermamx coverslips cell side up in freshly made Spurr's.

2) Following polymerization, remove coverslips by first sawing a small area of the epoxy/cell/substrate, then immersing in liquid nitrogen for a few seconds and prying away the substrate. The embedded cells are now on the surface of the epoxy.

3) Re-embed two fragments of the culture face to face for cross-sections, or cut the block parallel to the face for tangential sections.

We particularly like the round 13 mm Thermamox coverslips for immunoocytochemistry of cultured cells since they can be floated cell side down in a drop of 100 μL antibody - gold conjugate, which conserves reagents.

If a larger culture is desired, "Fermamox" culture dishes could also be used, which are equally resistant to chemicals common in TEM processing. These are also available through most EM and other suppliers.

Douglas R. Keene, Shriners Hospital Microscopy Unit, Portland, OR

Cornflaking Cells

In cytologic preparations for diagnostic cytology, cornflaking (aka the "brown artifact") is a common phenomenon, especially in Pap smears that contain many squamous epithelial cells. Caused by air trapped on the surface of superficial cells almost exclusively, these cells have a brownish crinkly appearance on their surface. It can range from a small area per cell to nearly the entire cell, and from a few cells per preparation (which is common) to nearly every cell. In the worst case, the preparation is useless and must be processed to remove the air.

Air bubbles form when xylene evaporates, leaving behind the exposed surface of these cells, which are covered by fine grooves to promote cell attachment. Anything that promotes xylene evaporation promotes cornflaking, such as prolonged draining between rinses, coverslipping multiple slides at a time, and coverslipping under a fume hood in which air enters the hood opening at 75 to 100 linear feet per minute.

To prevent cornflaking, coverslip one slide at a time. Do not drain the slide thoroughly. Try putting a chemical splash shield at the front edge of the fume hood and coverslip behind it. The shield diverts the incoming air and creates a quite zone where evaporation occurs more slowly.

While some recommend 50% glycine to remove the air, plain tap water works well. Since water removes the brown artifact, it is clear that this artifact does not occur before staining. Indeed, even if it did, it would be removed by the water rinses in the front half of the Pap stain and it would never be seen. Since tap water destains the orange G (OG) and EA dyes but not hematoxylin, restaining is required only for OG and EA.

It is not necessary to destain the slide, as the artifact is air and not stain. Remove the cover glass, pass the slide back from xylene to absolute alcohol, then to water. Microscopically check the slide to verify the removal of the artifact, then restain beginning in OG. A preceding rinse in alcohol is unnecessary.

Gary Gill, Diagnostic Cytology Laboratories, Inc.