

Preparing Gelatin Films on Coverslips

Tissue culture cells will attach to gelatin as good as plastic.
Use fishers gelatin it is less pure and will cut better.

5%-7% gelatin for EM

10% gelatin for LM

Make gelatin up in water. Heat to dissolve, make coverslips in a hot room if possible. This will allow the gelatin to stay thin enough to create a film on the coverslips.

Coat one side of the coverslip draw off excess and stand on edge to drain excess. Place coverslip in tissue culture petrie dish to let dry. Or use a small slide box.

Fix dry gelatin coated slides for at least 2 hours, or overnight at 4 degrees. Use 10% paraformaldehyde in PBS if the films will be used for LM. Use 4% gluteraldehyde if films will be used for EM. If cross linking is insufficient the gelatin will pull off when the cells grow. Warm to room temperature for one hour (to ensure good cross linking).

Wash in PBS to remove the fixative, 3-4X.

Transfer coverslips to a fresh dish, rinse five times five minutes each, with PBS. To get rid of bacteria, Rinse with culture medium. (plane DM medium without antibodies or serum, incubate One hour in incubator, then give a second rinse and incubate for 1/2 hour more.

Rinse with medium containing 10X antibiotics and fungicide for 1/2 hour. Give an extra rinse if you plan to culture for a long time, (> two days).

Rinse with medium 1X. Then culture your cells.

Protocol taken from the W.M. Keck Microscopy Facility at The Whitehead Institute website