**Cells in suspension**

**Basic protocol for cells in suspension for SEM**

(Incomplete)

1. Spin down cells. Suck off the supernatant media and wash in phosphate buffer. Spin down cells, suck off the supernatant buffer and cover with fixative. Fix cells in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer, pH 7.3-7.4 (or whatever the cells are happiest at) at 37 degrees (or whatever the cells are happiest at) for one hour.
2. Put cells into a syringe attached to a Swinex holder with nucleopore filter (size depends on size of cells).
3. Wash in buffer 0.1M cacodylate pH 7.3-7.4, 10 mls over 15 minutes.
4. Dehydration EtOH: 10 mls 30%, 50%, 70%, 95%, 100, 100, 100, 5 minutes each.
5. Dry: Either HMDS Or CPD
6. Mount, coat, view.

**Poly-L-Lysine adhesive for cell suspensions**

Poly-L-Lysine has been used with success for affixing various cell types to coverslips during processing for SEM. Two methods have been employed.

**I - As described by Mazia et al. 1975**

1. 0.1% Poly-L-Lysine (80,000 – 100,000 daltons) is dissolved in water.
2. The poly-l-ysine is dispersed over the surface of a coverslip.
3. The coverslip is washed with running water.
4. The coverslip is washed with a medium appropriate for the cell suspension.
5. The cell suspension is placed on the coverslip and the cell allowed to settle.

Once the cells are attached to the coverslip, fixation, dehydration and critical point drying may be carried out without risk of losing the cells.

**II - Modified version**

1. Poly-L-Lysine (MW 115,000) is prepared in phosphate buffered saline, pH 7.2.
2. A drop of polylysine is placed in the center of a 12 mm round coverslip ( a size which conveniently fits our Cambridge specimen stubs) and allowed to stand for one hour at room temperature.
3. Meanwhile, the cell suspension is fixed and rinsed in buffer.
4. The polylysine-coated coverslip is rinsed in running water.
5. A drop of washed cell suspension is placed at the point where the drop of polylysine had been.
6. The coverslip is then stored in a moist chamber for two hours or overnight allowing the cells time to settle.
7. The coverslip is dehydrated and critical point dried.

**References**

Mazia, D., G. Schatten and W. Sale. *Adhesion of cell to surface coated with polylysine*, Journal of Cell Biology [66(1):198-200](http://jcb.rupress.org/cgi/content/abstract/66/1/198) (1975)