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A Routine Schedule for the Preparation of Cell and Tissue Samples for Scanning Electron Microscopy

(From: Dykstra, M.J. 1993. *A Manual of Applied Techniques for Biological Electron Microscopy*, Plenum Press, NY)

1. Applications and Objectives: This technique can be applied to routine SEM samples of tissues, seeds, insects and other multicellular organisms, as well as cells grown on coverslips or membrane supports attached to filters of various types. In most cases, cells require special procedures to attach them to substrates that can be handled during processing, which will be described later.

The objective for preparing SEM samples is similar to that of TEM sample preparation. However, the level of resolution with SEM is generally considerably less than for TEM and we look at sample surfaces instead of looking through the sample as is done with the TEM. A well-prepared sample will have cell junctions preserved (no spaces between adjacent cells induced by processing), cell surfaces without excessive wrinkling or crushing artifacts, and dimensional aspects minimally changed.

2. Materials Needed:

McDowell and Trump's 4F:1G fixative (4F:1G)
0.2 M and 0.1 M Sorenson's sodium phosphate buffer, pH 7.2-7.4
100% ethanol for preparation of the dehydration series
2% aqueous osmium tetroxide solution (optional)

3. Procedure:

3.1. Prepare samples that can easily fit on the type of SEM stubs used for the particular SEM available (and which will also easily fit in the types of containers used with the critical point drying apparatus in the laboratory). Samples should be less than 2-3 mm thick wherever practical to lessen chances of bulk charging. Fix samples in

4F:1G fixative for 1-2 hr at room temperature. As with TEM samples, specimens may be stored in 4F:1G at 4° C for several years, if necessary. **For difficult (charging) samples, see below under Cautionary Statements .**

3.2. Rinse sample in distilled H_2O two times (5 min each).

3.3. Dehydrate sample:

50% ethanol 15 min
75% ethanol 15 min
95% ethanol 15 min
100% ethanol 15 min (2 times)

3.4. Critical point dry sample (or use alternative drying process employing dimethylsilizane or Peldri II as described later).

3.5. Mount samples on SEM stubs and sputter coat.

4. Results Expected: Samples will be dry and relatively stable under the electron beam, with minimal distortion and change in dimensional aspects.

5. Cautionary Statements: If a sample is likely to charge because it has a lot of sharp edges or pointed structures such as villi in small intestines and aerial sporangia in fungi, or is thick enough to present bulk charging problems (over several mm thick), rinse samples in 0.1 M Sorenson's sodium phosphate buffer pH 7.2-7.4 at room temperature two times (15 min each) and then post-fix in 1% osmium in the same buffer for 1 hr at room temperature. Then proceed to step #2 above. The cautionary statements concerning osmium and wash water containing osmium described under routine TEM preparation pertain.

Reference:

McDowell, E.M. and B.F. Trump. 1976. Histologic fixatives suitable for diagnostic light and electron microscopy. Arch. Pathol. Lab. Med. 100:405.