HIGH-RESOLUTION ELECTRON MICROSCOPY FACILITY STANDARD OPERATING PROCEDURES

The High Resolution Electron Microscopy Facility (HREMF) provides a resource to the scientific community at MD Anderson for high resolution imaging of cells, tissues, organs or polymers containing cancer agents. The facility is located at the Smith Research Building (South Campus) and houses a JEM1010 transmission electron microscope (TEM), a JSM 5900 scanning electron microscope (SEM) equipped with electron backscatter detector, a Technotrade coating system, a Leica Ultramicrotome, Leica Ultrastainer and other accessories needed to prepare samples for SEM and TEM. A technician with histology training is available to assist researchers in defining their specific needs related to SEM and TEM. Microscopes are equipped with digital cameras and CD burners, and are connected to a network printer and the Internet. The facility operates on a charge-back basis only for processing of samples and the number of microscope hours used to screen samples with technical assistance.

Getting Started

Investigators needing SEM and/or TEM contact Dr. Robert Langley by phone at 713-792-9142, or by e-mail at rlangley@mdanderson.org, to discuss feasibility of study. The procedure is as follows:

- A request form is sent to the investigator electronically, or it can be downloaded from the MDACC HREMF website.
- Dr. Langley, Mr. Kenneth Dunner, Jr., and the investigator discuss the logistics of sample preparation and detailed instructions (optional). Fixatives are prepared and picked up by the investigator just before samples are collected. Procurement of tissue samples from human or animal origin is the responsibility of the individual investigator. The investigators are required to fix all biological samples before they are brought to the facility.
- The samples are processed and pre-screened for quality of the preparation. The investigator is then notified that the sample is ready to be examined. We recommend that the investigator come and examine the samples with the assistance of Mr. Kenneth Dunner, Jr., at the microscope.
- Upon completion of the job request, the investigator signs the form, which is then submitted for subsequent billing. The charge-back system operates on an authorized fund transfer from investigator accounts to the HREMF account. Turnaround time for SEM results is approximately two weeks. Turnaround time for TEM results is approximately three weeks. Peer-funded investigators have priority. Services for investigators with no peer-funded grants are performed as time permits and the service is charged to the respective department.

Education

A detailed set of protocols is provided to the investigator regarding the preparation of samples for SEM and TEM. In the majority of cases the investigator fixes the sample with fixative prepared by HREMF staff and HREMF staff performs subsequent processing. Screening of samples with the assistance of technical support is recommended at all times. Background references on the particular investigation are usually requested by HREMF to ensure that the correct fixatives and specific information regarding the specimen is known before the analysis. The staff is prepared to offer

technical advice and assistance for more complex imaging analysis (*e.g.,* double-immunogold labeling, etc.).

Equipment

Equipment used by this facility are as follows:

- JSM 5900 scanning electron microscope (JEOL USA, Inc., Peabody, MA) equipped with backscatter electron detector and digital camera
- JEM 1010 transmission electron microscope (JEOL USA, Inc., Peabody, MA) equipped with a digital camera
- Ultramicrotome (Leica Microsystems Inc., Deerfield, IL)
- Ultrastainer (Leica, Microsystems, Inc., Deerfield IL)
- Vacuum Coating System (Technotrade International, Inc., Manchester NH)

Materials and Methods

General SOP Guidelines for Electron Microscopy Samples

1. Primary Fixation of Samples

a) A solution of 2% paraformaldehyde and 3% glutaraldehyde is recommended as a primary fixative for electron microscope samples. Concentrations of glutaraldehyde below 2% may result in extraction, whereas as shrinkage may occur when using concentrations above 4%.

b) Tissues should be removed from the animal as quickly as possible postmortem and immersed in the primary fixative during dissection into EM-sized pieces.

c) The tissue samples for EM should be no more than 1-mm thick in at least 1 mm in dimension.

d) The fixative solution should be approximately 5 times the volume of the sample.

2. Trimming and Orientation of TEM samples

Materials Needed:
Paraffin-filled Petri dish
New single-edge razor blades
Applicator sticks with 1 end shaved down to a flat surface
4-dram specimen vials
EM fixative
Pipets

Procedure:
Necroscopy: During necroscopy, quickly cut off a small piece of tissue several mm in thickness and place it into a large droplet of fixative on the surface of the paraffin in a Petri dish. Slice the tissue sample with a razor blade into pieces 1 mm in thickness. Pick up the 1 mm thin slices by touching them with the

flattened end of an applicator stick, and then place them into the vials containing about 1 ml of EM fixative.

Core Grant Citation

We request that publications using the HREMF include an acknowledgment of the Cancer Center Core Grant CA16672. Two copies of the publication acknowledging the Core grant should also be submitted to the facility at 1515 Holcombe Boulevard, Unit 173, Houston, TX, 77030.