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Sample preparation in TEM

The process of specimen preparation in TEM involves the following steps:

Fixation: Fixation of the specimen stabilizes the cell so that further change or damage to the cell will not happen. Through this process, the sample is preserved to give a snapshot in time of the living cell. Fixation can be done through two methods as follows:

a. **Chemical fixation:** This method is used for stabilizing biological samples. Chemical substances are used to cross link protein molecules with nearby molecules. Chemical used in this method is glutaraldehyde.
b. **Cryofixation:** This method involves rapid freezing of the sample in either liquid nitrogen or liquid helium. The water content in the sample thus gets transformed into a vitreous ice form.

Secondary fixation: To increase the contrast of the minute structures inside the specimen and give more stability, a secondary fixation is carried out using osmium tetroxide (OsO_4). Without inducing any change in the features of the structure.

Dehydration: Freeze drying, or dehydration, of the specimen is the process by which the water content in the specimen is replaced with an organic solvent. Ethanol and acetone are the frequently used solvents in this method. Dehydration is important as the epoxy resin used in further steps does not mix with water.

Infiltration: In infiltration, epoxy resin is used to penetrate the cell, which will then occupy the space and make the sample hard enough to bear the pressure of sectioning or cutting. This process is also called embedding. The resin is then kept in an oven at 60° overnight to allow for setting. This process is called polymerization.

Polishing: After embedding, some materials are subjected to polishing. Polishing a specimen reduces scratches as well as other problems that can minimize the quality of the image. Ultrafine abrasives are used to give the specimen a mirror-like finish.

Cutting: For study under an electron microscope, the sample should be semi-transparent to allow the passage of electron beams through it. To achieve this semi-transparent nature, the sample is sectioned into fine sections using a glass

or diamond knife attached to a device known as ultramicrotome. The device has a trough that is filled with distilled water.

The sections cut are collected in this trough and are then moved to a copper grid to be viewed under the microscope. The size of each section should be between 30 nm and 60 nm to get the best resolution.

Staining: Staining in specimens is usually done twice – before dehydration and after sectioning. In this process, heavy metals like uranium, lead, or tungsten are used to increase the contrast between different structures in the specimen, and also to scatter the electron beams.

Staining before hydration is done in block, while in staining after sectioning, the sample is exposed briefly to an aqueous solution of the above metals.

A cryofixed specimen may not undergo all these procedures. It can be directly subjected to cutting and then shadowed using vapors of platinum, gold, or carbon before visualization under the TEM.

JEM-ARM200F-G TEM