

Preparing Biological Tissues for TEM Study

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Introduction: Chemical fixation is a widely-used method for preparing biological tissues for fine structural study with the electron microscope. This type of fixation may be applied simply by immersing small pieces of the specimen in the fixative of choice. This procedure is simple, rapid, and yields acceptable results. However, the method preferred by many microscopists is that of whole-body perfusion which utilizes pressure to rapidly deliver the fixative through the vascular system of the animal. Vascular perfusion, while more laborious, is known to yield optimal and complete tissue preservation as the perfusate is distributed uniformly to all organs and tissues by the vascular channels.

Choice of Fixative and Buffer System: Many microscopists over many years have studied the fine structure of biological tissues. It has become established protocol that biological materials react best to chemical treatment with glutaraldehyde, or a combination of glutaraldehyde and paraformaldehyde, followed by post-fixation in osmium tetroxide. Primary vascular perfusion with 3% glutaraldehyde, buffered with 0.2M sodium phosphate, or another comparable buffer system, to a pH range of 7.2-7.4, followed by immersion fixation in similarly buffered osmium tetroxide, is a proven and thoroughly tested method.

Post-fixation Washing of Tissues: It is necessary to wash/rinse the specimen following primary aldehyde fixation and before subsequent post-fixation with osmium tetroxide. This step removes traces of aldehyde that would contaminate the tissue with a precipitate formed between the aldehyde and osmium during the secondary fixation step.

Dehydration of Tissue: Embedding media utilized to infiltrate biological tissues are not miscible with water. Therefore, it is necessary to dehydrate the specimen by passing it through a series of solutions to the point where the tissue is fully miscible with and thus may be infiltrated with the embedding medium. Ethanol is the dehydrating agents commonly used and preferred for this procedure.

Infiltrating and Embedding Methods: Once the tissue is thoroughly dehydrated, it is next subjected to infiltration by the embedding medium of choice. Infiltration is followed by embedding and polymerization for a period of several hours. This process then yields a completely hardened block that can be thin-sectioned for ultimate study. Infiltration may be long or short. Since most epoxy embedding media are by nature very viscous, prolonged infiltration times of several hours would not appear to be necessary, since the medium will harden soon after initial mixing. In order to reduce the viscosity of the embedding medium and improving infiltration, specimens may be placed in an intermediate solvent combination of propylene oxide and the embedding medium for a prescribed period. However, this step may be eliminated. Polymerization overnight at 60°C, rather than with increasing temperature over a period of two nights as suggested by earlier techniques, is adequate to produce the hard blocks.

Standard Processing of Biological Tissues for TEM Study

- 1) *Primary fixation by whole-body vascular perfusion:* 3% glutaraldehyde for general fixation or a combination of 2% glutaraldehyde and 2% paraformaldehyde for maximum penetration. Fixative is buffered with sodium phosphate buffer to a range of pH 7.2-7.4.
- 2) *Washing:* Specimen is washed for 1-2 hours in sodium phosphate buffer wash with two or three changes. Thorough washing is necessary to remove traces of aldehyde which may react with osmium ions and cause a precipitate to occur over the tissue sections. Sucrose may be added to the phosphate wash in order to maintain optimal osmolarity.
- 3) *Secondary fixation by immersion in 1% osmium tetroxide:* Post-fixation is achieved by placing small cubes of the tissue in similarly buffered cold osmium tetroxide. Thirty to forty-five minutes usually is sufficient duration for this secondary fixation.
- 4) *Post-fixation rapid rinse:* Two rapid rinses, 1-2 minutes each with constant swirling, are necessary to remove excess osmium which could react with the dehydrating agent.
- 5) *Dehydration:* Increasing concentrations of cold ethanol. Ten to twenty minutes each in 50%, 70%, 95% and 100% ethanol. Two additional changes in 100% ethanol while tissue reaches room temperature. The transitional solvent propylene oxide is omitted in this procedure.
- 6) *Initial infiltration:* 1:1 solution of ethanol and embedding medium for 30 minutes on a rotator. At this time, specimens should sink to the bottom of the vial. Additional 20-30 minutes of initial infiltration in fresh 1:1 solution may be used.
- 7) *Final infiltration in epoxy embedding medium:* Three – six hours is sufficient but, if desired, the tissues may remain overnight in full epoxy embedding medium.
- 8) *Polymerization:* Specimens are placed in appropriate molds, such as Beem capsules. Polymerize in oven at 60°C – 70°C for overnight period, or about 15 hours. Blocks may be sectioned the following morning and polymerized further if necessary.

The above procedure is very simple and has been used successfully for many years for processing soft organ tissues, including heart, liver, kidney, muscle, pancreas, lung, endocrine glands, skin and others. Some processing schedules usually call for prolonged infiltration times when using epoxy media. However, these media are comprised of individual components that are relatively viscous. When combined, the full medium takes on the character of the individual components and likewise is quite viscous. Even low viscosity alternatives will harden soon after preparation. Consequently, it is not advantageous time-wise to infiltrate tissues for long periods of time since infiltration probably will occur in the first few minutes when the medium enjoys the most fluidity. Most procedures also call for a transitional solvent, usually propylene oxide, in combination with the medium. The rationale is that propylene oxide reduces the viscosity of the medium and improves infiltration. However, ethanol will reduce the viscosity of the embedding medium sufficiently to enhance infiltration. The transitional propylene oxide – embedding medium step routinely is eliminated in this procedure.