Microsc. Microanal. 8 (Suppl. 2), 2002
DOI 10.1017.S1431927602107252

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Processing Cell Cultures, Cytospins, Smears and Epoxy, Paraffin or Frozen Sections on Glass or Polystyrene Supporting Substrates for TEM: A Review

K. Chien, M.L. Heathershaw, R.C. Heusser, H. Shiroishi, R. Gonzalez, C.C. Nast and A.H. Cohen
Dept. of Pathology & Laboratory Medicine, Cedars-Sinai Medical Center, Los Angeles, CA 90048

Glass slides (or coverslips) and polystyrene Petri dishes are the most suitable and commonly used supporting substrates for the growth of cell cultures. Glass slides are also widely used in cytology, histology, hematology and the EM laboratory for supporting substrates of cytospins, smears and epoxy, paraffin or frozen sections. Sometimes these specimens needed to be processed for TEM examination. One major difficulty is the separation of polymerized epoxy blocks from the supporting substrates. (Embedding medium Vestopal W is more easily separated from glass surfaces but less commonly used today). Thermo-shock by dry ice or liquid nitrogen has been used with some success but the results are inconsistent. Numerous techniques have been developed to overcome this separation problem by coating glass slides with Teflon, silicone and carbon etc., or by placing sections on other materials such as Mica, Silicone rubber, polyethylene or aluminum dishes. However, these substrates are either unsuitable for cell growth or not transparent for examination by light microscopy. This abstract combines various methods and demonstrates simple, rapid processing procedures for this task.

The most common dehydrating agents for biological specimens in TEM are alcohol and acetone. Acetone is a stronger solvent than alcohol, however, alcohol is only miscible with epoxy resins. It is a poor vehicle to carry resin into dense specimens and may cause uneven hardness of the blocks. Thus, propylene oxide is used as a transitional fluid in routine embedding. Glass slides and coverslips are insoluble in these organic solvents and no special precaution is needed during processing (except breakage). Polystyrene Petri dishes are more susceptible to be dissolved by acetone, propylene oxide and plastic resins (at high temperature). In the situation of embedding thin-layer specimens on polystyrene, epoxy resin can be mixed directly with alcohol for optimal infiltration and eliminate the use of propylene oxide. The sectioning quality of the blocks is comparable and ultrastructural morphology is even better preserved. Cells are infiltrated with 100% resin for an hour before final embedding. The tips of polyethylene BEEM capsules (00 size) are cut off with a Teflon coated razor blade and the lid is placed on the cutoff end. This is done because the original rim of the capsules is more flat and smooth then the fresh cut end. The capsules are filled with epoxy resin and inverted onto the specimen. Excess resin around the base of the capsules need not to be wiped off because polyethylene capsules will not polymerize to the resin as happens when gelatin capsules are used. The epoxy resin is slowly polymerized in 40°C oven for 2 days and then raised to 60°C for 2 days or 70°C overnight to complete the polymerization. An initial low temperature causes the resin to be partially polymerized with the specimen but does not dissolve, infiltrate or polymerize with the Petri dish. After polymerization, Petri dishes are removed from the oven and rapidly cooled down to room temperature with running tap water. The Petri dish is then placed on a hot plate at approximately 100°C for 20 to 40 seconds. Each BEEM capsule can be gently "peeled off" from the Petri dish by gradually increasing pressure. If the first attempt fails, do not force separation but continue to heat for a few more seconds and then re-try. After separation, the monolayer cells will be captured just beneath the shinny surface of the blocks.[1,2] High temperature for a short time period temporarily softens a thin layer of epoxy resin allowing easy separation. Prolonged heating must be avoided because the breakage will occur in the softened

epoxy block instead of at the interface with the supporting substrates. If the temperature is not sufficiently high enough, the epoxy blocks will be forced to "pop off" the supporting substrate which will cause an uneven flatness of the block face. By re-heating the block on a hot plate, the resin will expand and retain flatness of the block face. If the Petri dish does polymerize with the epoxy blocks, it can be dissolved away with xylene but the block face will not be smooth making a diamond knife approaching and sectioning more difficult.

After demonstrating this heat separation method in the People's Republic of China, an interesting modified method was developed. Some microscopists could heat the glass slide using a lighter and knock off the epoxy blocks with the flick of a finger. Without knowing the exact temperature and time, this technique can be simply applied with experience.

When cell cultures or sections are on a thin and fragile glass coverslip that may fracture during heat separation. A modified method can be used during final embedding. The coverslips with a small amount of resin on the backside are placed on top of a glass slide. BEEM capsules filled with resin are then placed on top of the cell monolayers and polymerized in a 70°C oven overnight. The coverslips will be polymerized to the glass slide that provides extra needed support so that breakage will not occur during heat separation. The timing and the amount of peeling force are much more critical with this technique.

Since both ends of the blocks are flat, they can be placed on a glass slide with the cell-side up and examined under a light microscope. The length of the epoxy block (about 8mm) increases the distance between the specimen and the condenser lens but optimal photographs can be obtainedeven with an oil immersion lens. Once an area of interest is selected, the epoxy block can be heated on 100°C hot plate for about two minutes and easily split in half with a Teflon coated razor blade. The portion of block containing the area of interest can be divided again thus saving ¾ of the block for future sectioning if needed. All three pieces are placed into a standard size block holder with the block of interest raised slightly above the others. It can then be trimmed and sectioned horizontally at the plane of cell growth. Since the block face is flat and shinny, a diamond knife can be precisely aligned and the first few sections which contain the largest portion of the cell can be immediately obtained.

For vertical sectioning at the plane of cell growth, the cell monolayer will be at one edge of the section and a formvar-coated grid is needed. By adding a small amount of 100% epoxy resin between two block faces and rapidly polymerizing in 100°C oven for 2 hours, two monolayers can be re-embedded into one block. By re-orientation, each ultra-thin section will contain two monolayers and no supporting film is needed.

In conclusion, the ultrastructural pathology and specific of staining properties on various specimens that were not originally processed for TEM can be re-evaluated. [3,4,5,6,7,8]

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https://doi.org/10.1017/S1431927602107252 Published online by Cambridge University Press