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Lab-Tek Chamber Slide for TEM Prep: A Simple, Rapid, and Reliable Protocol for *In Situ* Embedding Monolayer Cell Cultures in Epoxy and LR White Resin

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Introduction

Preparation for TEM of resin-embedded monolayer cell cultures usually requires scraping cells from the culture substrate before or after primary fixation, which disturbs the monolayer and often results in mechanical trauma to the cells. After harvest, the cells are centrifuged and processed as a pellet in a micro-centrifuge tube through a prolonged procedure of post-fixation, dehydration, infiltration, and finally embedding to ensure a "well done" sample block for subsequent sectioning, staining, and observing under a transmission electron microscope. Other disadvantages to this method include the loss of cellular orientation and information on cellular interaction, as well as difficulty in collecting a large quantity of cells to form a sizable pellet for processing, which is of special importance when samples are differentiated cells and neurons cultured in low density. In an alternative method, cells can be seeded on either glass or plastic cover slips or Petri dishes, and then processed and embedded directly as a monolayer. After polymerization, however, one frequently encounters difficulties separating the coverslips or Petri dishes from the surface of the polymerized resin in which the cells are embedded. The surface of the embedded monolayer is often damaged or contaminated by debris during the process of the removal of the monolayer from the support substrate. In several projects where cell orientation (apical *vs*. basal) was critical and intercellular relationships as well as extracellular matrix were studied, we have developed a new protocol in which Lab-Tek chamber slides were used to *in situ* embed monolayers in epoxy and LR White resin. The current protocol is simpler and less time-consuming than the conventional methods, and the embedded monolayer can be easily separated from support substrate simply by peeling off the slide from the chambers by hand.

Experimental Protocol

Lab-Tek plastic Permanox™ slides containing 2-, 4-, or 8-chambers were used (Fig. 1a). The chamber slides were purchased from PGC Scientifics (Cat. No. 81-6669-18, 81-6669-21, or 81-6669-24; also available via Electron Microscopy Sciences). Cells of differing densities were seeded in coated or non-coated chambers, depending on the culture conditions specifically required for the cells.

A general protocol we used for embedding different types of cells in epoxy resin is as follows.

1 Rinse the aldehyde-fixed monolayer in a chamber slide three times with 0.1 M cacodylate buffer, pH7.4 and post-fix cells with buffered 1% osmium tetroxide for 30 min at room temperature in a dark box.

2 Rinse three times with the same buffer followed by two quick changes with Millipore water.

3 *En bloc* stain the cells with 2% aqueous uranyl acetate for 30 min in the dark.

4 Rinse two times with Millipore water and then dehydrate cells with a graded ethanol series (70%, 90%, and two times 100%) followed by two changes in 100% acetonitrile, 5 min each.

5 Infiltrate cells with a mixture of 50% acetonitrile and 50% Eponate12/Aradite resin (Ted Pella; see formula in Ref. 1) for 1 hr followed by two changes in 100% resin of same formulation, 1 hr each.

6 Embed the cells by adding fresh epoxy resin up to a little more than half (~7 mm) of the well. Place the chamber slide in a 70°C oven to allow the resin to polymerize overnight (Fig. 1b). *The whole protocol for processing the monolayer takes less than 5 hours.*

7 Take the chamber slide out of oven; clearly mark the chambers before separating them from the slide if different cells or conditions are used. Peel off the slide from the chambers by hand (It should be easy; Fig. 1c). Note: touching or bumping the clean surface of the resin embedded monolayer beyond this point will contaminate or damage that part of the sample.

8 Locate "good culture" by reflecting the surface of the embedded monolayer under a dissecting microscope and use a fine Sharpie or diamond scriber to draw circles around them. An experienced eye can usually tell the "good culture" spots as

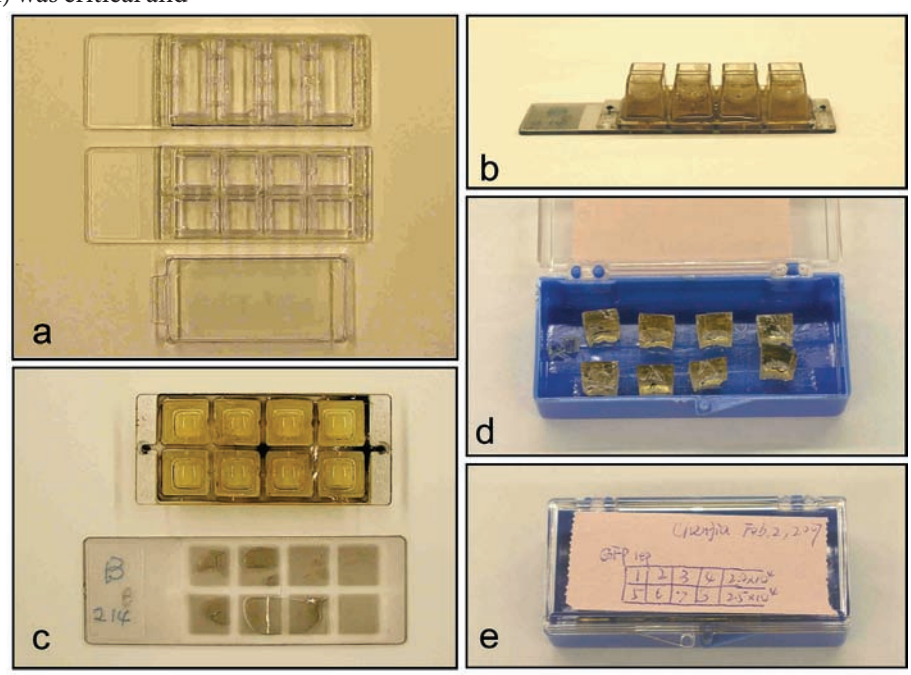
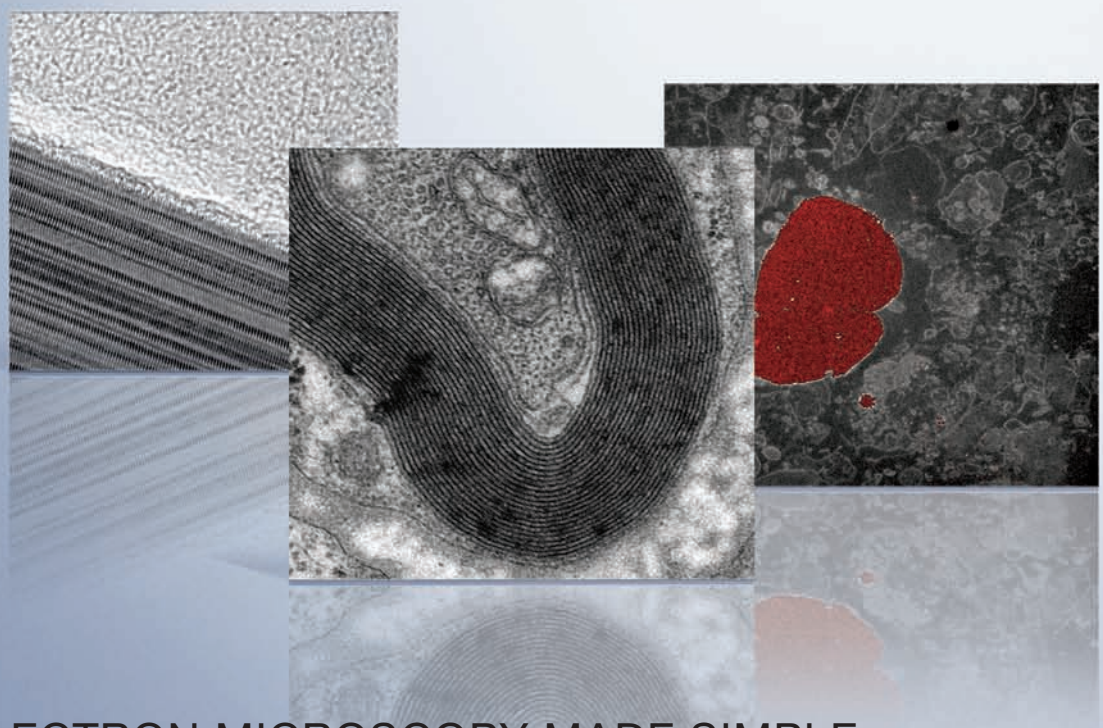


Fig. 1. Lab-Tek chamber slide. a, 4- and 8-chamber slide and lid; b, polymerized resin in chambers. Note: 2/3 of the chambers are filled with resin; c, chambers and detached slide; d, sample blocks set on double-sided tape in a sample box with surface of monolayer facing up; e, sample box with labels.



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a group of cells with the right density and with sharp and clear cell profiles in an area of clean surface.

9 Clamp the chambers in a vise and cut off the plastic sides of chambers with a razor blade. Be very careful not to cut yourself - it can be a bad injury. Mount plastic blocks on strips of double-sided tape in a sample box for storage (Fig. 1d&e).

10 Chip or saw off the circled areas of "good culture" with a razor blade or jeweler's saw. Screw the block into a sample holder, trim, and section it in an ultramicrotome.

The following protocol was used to embed cells in LR White for immunoelectron microscopy.

11 Do Steps 11-13 on ice. Quench the fixed cells with 0.15% glycine in 0.1 M phosphate buffer, pH7.4 for 5 min, then rinse three times with the same buffer.

12 Dehydrate the monolayer with 50%, 70%, 90%, and 100% ethanol, 5 min each.

13 Infiltrate cells with 1:1 mixture of 100% ethanol: 100% LR White for 40 min followed by two changes of 100% LR White, 40 min each.

14 Fill up 2/3 of the wells with 100% LR White and put the lid on the chambers. *The total time for processing the sample is less than 3 hrs.*

15 Place the chamber slide at 0-4 °C to polymerize with UV light for 3 days or at 64 °C for 24 hrs. After the resin is completely polymerized, use the above procedure (Steps 7-10) to separate the chambers and slide and to prepare the blocks for ultramicrotomy.

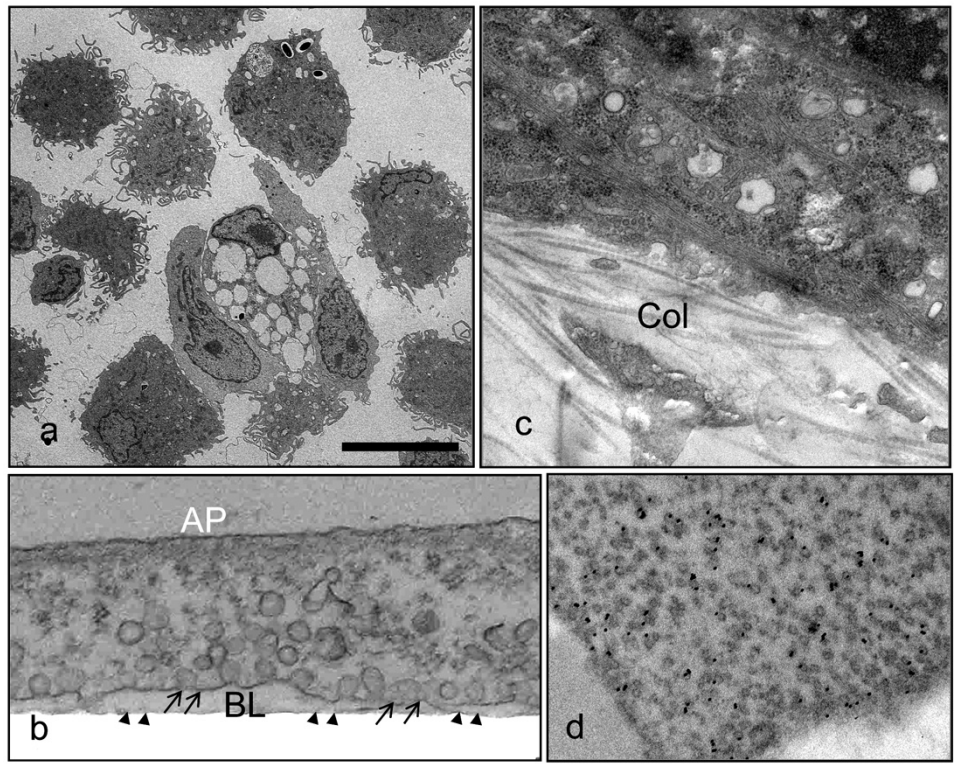

Fig. 2. TEM micrographs of monolayer samples embedded in Lab-Tek chambers. a, horizontal/transversal (en face) section of cells; b, vertical section; Note: the cell's plasma membrane (arrows) at the basal side remained intact and is covered by a small amount of resin from outside (arrowheads), after separation from the slide. c shows a cell and its relationship with the extracellular matrix, such as collagen fibers (Col); d, immunogold-labeled caveolin 1 in a LR White embedded cell. AP, apical side; BL, basal side. Bar= 10 μm in a, 0.4 μm in b, 1.0 μm in c, and 1.0 μm in d.

Result and Discussion

As shown in Fig. 2, the morphology of the cells processed in this manner resulted in good quality for analysis. The extracellular matrix shown in Fig. 2b was well penetrated by resin and there were no difficulties sectioning the block. Although the majority of the sections in our study were made in a horizontal/*en face* direction (Fig. 2a, 2b, and 2d), it is possible to obtain vertical/cross sections also (Fig. 2c). We noticed that the vertical/cross sections showed intact plasma membrane along basal side of the cells, demonstrating that separation of the chambers from the slide did not cause any tearing of the cells. In LR White embedded samples, the resin was completely polymerized and easy to section. Specific labeling with immunogold indicates that a successful sample preparation was achieved (Fig. 2d).

This protocol provides an easy solution for *in situ* embedding cultured cells in resin. The protocol is less time-consuming and labor intensive than conventional methods, and is reliably completed by experienced EM technicians within 3-5 hrs. We are aware that in a recent methodological book John Bozzola detailed using Lab-Tek chamber slides for *in situ* embedding monolayer cultures in epoxy resin (2), but the protocol takes a much longer time to complete. We recommend using acetonitrile instead of acetone for infiltration, because acetone will dissolve the plastic chamber. This method also provides possible applications for correlative microscopic studies of the same cells with optical and electron microscopy. Several cons, however, must be considered: ultramicrotomy of these samples requires advanced skills and may not be suitable for beginners; separating the chamber walls from the block using razor blades is also fraught with danger and should be done with extreme care, and finally, freeze-substitution protocols may not be possible because the chambers and slides may separate prematurely due to abrupt temperature changes ■

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References

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