TECHNICAL NOTE:

SERIAL SECTIONS WITH PLASTIC-EMBEDDED TISSUE

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ABSTRACT

For embedding, sectioning and examination of small samples of tissue such as (but not limited to) the ovaries of insects, plastic embedment offers considerable advantages over the more classical paraffin wax embedment. However, in many cases it is hard to maintain plastic sections in serial order. Here we describe a simple method, which overcomes this problem.


INTRODUCTION

The purpose of this note is to acquaint tissue and cell biologists who have not had experience in plastic embedding with the advantages in tissue quality, convenience and simplicity of this technique. When tissue samples to be examined are inherently small, plastic embedment offers significant advantages over paraffin embedment. For instance, internal organs of many species of insects may themselves be nearly microscopic in size. The ovaries of a female Drosophila are only 600 μm long, 250 μm wide, and the male testes are even smaller. Attempts to embed any such organs or tissue samples of similar size in paraffin can be frustrating. Such small samples cannot easily be followed, placed, nor oriented as molten paraffin is added to a mold. Presently, embedding materials including molds, resins, and hardening agents, all specifically intended for light microscopy examination of tissue, have been developed from those used for preparation of tissue to be examined by transmission electron microscopy. With plastic embedment, polymerization of the transparent resin is sufficiently slow to allow the sample to be carefully positioned and oriented after it has been placed in activated resin within a mold, and this can even be done under a stereomicroscope. Tissue may then be successfully cut from blocks with a face of 2 mm² or smaller. Furthermore, while paraffin embedment works well for examination of whole tissues, plastic embedment is far superior if cellular details need to be preserved.

One drawback often encountered with plastic embedment is difficulty in keeping successive sections in serial order. With paraffin embedment, seriality of successive sections is easily achieved, as the trailing edge of each paraffin section tends to adhere to the leading edge of the next, forming long ribbons. When plastic blocks are cut, the sections do not form ribbons and thus each section often needs to be individually transferred to distilled H2O on a slide or warming bath. There they will spread and flatten. However, upon being placed on the surface of a H2O bath, as sections flatten and decompress, we find that they spin rapidly and move over the H2O surface in a random manner. Thus, sequential sections may come to rest far from each other. Nor can sections be easily manipulated via probe, forceps, or any other such tools. When touched by even glass probes, the sections tend to fold and/or adhere to the probe. As the H2O evaporates, sections dry against the ETOH-cleaned glass surface of the slide, but are distributed randomly rather than in sequential order (Figure 1A).

In the past, several strategies have been employed to avoid this problem. One solution was to create slides with small wells into which the sections could be manipulated (Aweele, 1976). Royer (1988) suggested coating the “leading” and “trailing” surfaces of each block with contact cement, causing the sections to adhere to each other. Alternatively, a water-filled trough attached to the microtome blade has been used to receive and trap the sections, much as would be done for EM thin sections (Wali and Jagadeesh, 1988). When sections were subsequently to be subjected to immunohistochemical staining, Guitteny et al. (1988) recommend placing each section in a separate vial. In two additional methods, loss of seriality was not a problem because of the size of the sections; in one case sections were 5cm × 5cm (Randall et al., 1988) in another instance the sections were cut 25 μm thick and then examined via confocal scanning microscopy (Tosney and Landmesser, 1986).

MATERIALS AND METHODS

JB-4 resin system, molding cups, and block holders were purchased from Electron Microscopy Sciences (Hatfield,
Individual sections were cut from the block they were placed sequentially, each in its own drop (Figure 1B). The water was allowed to evaporate on a warming tray (Figure 1C). At 40°C the water dried and sections adhered in about 10 min. while several sections in a single large drop required over 30 min. Schoenwolf and Chandler (1983) mention a similar strategy in a paper focused primarily upon the mechanics of plastic embedding. A 24 × 60 mm cover slip was added and the sections were examined with phase contrast optics. If tissue has been pre-stained, or contains fluorophores, or is to be examined with phase contrast optics, there is no need for post-section staining. Yet resolution of small details of tissue and intracellular structures remains excellent.

RESULTS

As an example, we have used insect ovarian follicles, each consisting of an oocyte and surrounding epithelial cells; in many species, these occur in long “chains” called ovarioles. In each ovariole there are sequentially positioned follicles, each older than the one immediately proximal to it. It is often desirable to cut such ovarioles longitudinally, making position in the mold of great importance. Figure 2 shows sequential sections of two plastic embedded insect ovarian follicles sectioned 4μm thick, and photographed with a 20X, 0.45 N.A. Phase Contrast objective.

DISCUSSION

There are several advantages favouring embedding of small samples in plastic. First, the position and orientation of the tissue can be followed throughout the process. Second, preservation of tissue and intracellular structural details are preserved far better than when embedded in paraffin. Third, by the strategy described here, seriality of sections is easily preserved. Fourth, drying time by which the water-floated tissue becomes adherent to the slide is greatly reduced.

In paraffin embedding, assurance of position and orientation of very small specimens is nearly impossible. With plastic embedding, the resin does not harden at once, but remains both fluid and transparent after surrounding the specimen. Thus, the ability to position and re-position a specimen; even to determine this with a stereomicroscope, is a major advantage. For small specimens, the combination of ease with which the specimens could be positioned and the clarity with which cellular level detail was preserved demonstrated that this was a clearly superior method of preparation.

Stereological measurements in which z-axis measurements depend upon accurate examination of serial sections require that the order of sections on the slide be maintained. By placing each successive section in its own drop, this was assured. The series of sections shown in Figure 2 demonstrate the preservation of seriality easily achieved with this technique.

While “East-West” orientation of sections is maintained,
this is not true for “North-South” orientation. However, micrographs of sections can be rotated to a common orientation, as was done here, and the “left-right” positions remain consistent and correct. Alternatively, the camera may be rotated, allowing the sections to be studied in consistent orientation on a monitor.

The case with which sections can be confidently identified in their proper sequence is the most useful feature of placing them in separate small drops. Occasionally, when cutting sections 1–5 μm thick, a section may be irretrievably lost; and this can easily be recorded during the process of sectioning a block. The precise section and where it would have appeared in serial sections can thus be included in the data. Stereological measurements require accurate 3-D information (Howard and Reed, 1998), and it is for such measurements that this technique would be of particular importance. In such cases, the procedure described here is simple, rapid and effective.

REFERENCES


