Technical methods

A replica technique for paraffin sections for transmission electron microscopy

LILIAN LIM *From the Electron Microscopy Laboratory, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia*

Although Lehy and Zeiton (1970) used ultrathin, epoxy-re-embedded paraffin sections for their studies of microadenoma, I am not aware of any other attempts to use a replica technique utilizing paraffin sections for transmission electron microscopy.

The method described has been used to examine at the electron microscopic level the appearance of damaged collagen fibrils in chemically treated rabbit tendons. This procedure allows for the extension of routine light microscopic information to a three-dimensional level at electron microscopic resolution without the need for elaborate reconstruction methods for the serial sections. A paraffin block of which stained sections have been found to contain lesions by light microscopy was sectioned again for replica electron microscopy and ultramicrotomy after being embedded again in epon. Thus it was possible to examine the same lesion for light microscopy, conventional thin-section electron microscopy, and replica electron microscopy.

Shadowing and Replication

For more detailed description on replica technique procedures, the reader should refer to Bradley (1965) and Pease (1965).

TENDON

1 Preshadow deparaffinised sections with chromium at an angle of about 45 degrees.

2 Replicate with thick layer of carbon. The replica disintegrates easily in the later stages of its processing if the carbon layer is insufficiently thick.

STRIATED MUSCLE

1 Preshadow with chromium at an angle of about 20 degrees.

2 Replicate with thick layer of carbon as for tendon.

Received for publication 13 August 1972.

The replica is then made to adhere to unfilmed grids with 0.5% formvar in ethylene dichloride and simultaneously cleaned and removed from its glass substrate with bleach. The formvar is dissolved away with ethylene dichloride or chloroform before electron microscopy.

The replicas were examined and photographed with a Hitachi HS-8 electron microscope.

Simultaneous Separation and Cleaning of the Replica

- 1 Place unsupported grids over the replica.
- 2 Carefully flood 0.5% Formvar in ethylene dichloride over the grids. The Formvar serves as adhesive for the replica to the grid.
- 3 Blot off excess with filter paper.
- 4 Place in desiccator for at least 10 minutes.
- 5 Scrape round the perimeter of each replica grid with a sharp scalpel blade.
- 6 Immerse in Kirox (Clorox or other commercial sodium hypochlorite).
- 7 After three min gently try to ease replica grids away from slide with a sharp needle. Do not use force if grid does not budge but wait for another 2 min and try again.
- 8 Overturn loosened grids to expose replica fully to bleach for another 10 minutes.
- 9 Rinse well in three changes of distilled water.
- 10 Dry grid by blotting off excess water from its edge with a piece of filter paper.
- 11 Check under light microscope to see if replica is cleaned. Repeat steps 8-11 if necessary.
- 12 Dry the clean replica grids in a desiccator for at least 15 min before proceeding.

Removal of Formvar from Replica

- 1 A replica grid is placed replica-side up over a small piece of degreased pre-cleaned wire gauze in a cavity block.
- 2 Ethylene dichloride is gently pipetted drop by drop till the meniscus just touches the lower surface of the grid. At this point stop for a few seconds.
- 3 Continue to add the solvent drop by drop while taking care not to let the drops fall directly onto the replica.
- 4 When the replica grid floats up, stop adding the solvent and leave for 30 min or longer.
- 5 Pipette off solvent gently until its meniscus is well below the wire gauze.

Fig. 1 *Replica of normal collagen fibrils from rabbit tendon sectioned obliquely. $\times 28\,600$.*

Fig. 2 *Replica of normal rabbit collagen fibrils. l.s. $\times 132\,000$.*

Fig. 3 *Replica of normal rabbit skeletal muscle. l.s. $\times 20\,900$.*

Fig. 4 Replica of normal rabbit skeletal muscle. *l.s.*
× 28 600.

6 Lift up the wire gauze containing the replica grid and put it onto a clean glass slide.

7 Check under the light microscope to see if replica is clean. Repeat steps 1-7 if necessary. When clean the replica is ready for electron microscopy.

Comment

I have used dilute hydrofluoric acid to separate the replica from the slide, but found that unless the replica is separated almost as soon as it has been replicated, the replica becomes increasingly adhesive to the glass surface. With the simultaneous separation and cleaning method, modified from that of Cox, Gilchrist, and Juniper (1970), it has been possible to separate the replica even after it has been stored for three weeks. The Formvar, which has been used as a temporary adhesive between replica and grid, can be dissolved away by chloroform instead of ethylene dichloride. Replicas appear darker when chloroform is used, and it may be that the Formvar is not completely dissolved by this reagent under the conditions employed.

This replica technique has so far been applied only to tendon and striated muscle as it reproduces banded and filamentous components quite faithfully as illustrated by Figures 1, 2, 3, and 4. This method might also work for decalcified bone.

I am grateful to Professor K. S. Lau, the Head of the Electron Microscopy Laboratory, for providing the facilities, Dr J. Koehler and Mr K. L. Chong for advice and help, and Dr P. Balasubramanian for supplying the experimental material. I also wish to thank the histopathology laboratory staff for their cooperation.

References

- Bradley, D. E. (1965). Replica and Shadowing Techniques. In *Techniques for Electron Microscopy*, 2nd ed. Blackwell, Oxford.
- Cox, G. C., Gilchrist, A. J., Juniper, B. E., and Williams, P. R. (1970). *Techniques for Plant Electron Microscope*. Blackwell, Oxford.
- Lehy, T., and Zeitoun, P. (1970). Utilization of paraffin embedded material for electron microscopy. *Lab Invest.*, 23, 52-57.
- Pease, D. C. (1965). *Histological Techniques for Electron Microscopy*, 2nd ed. Academic Press, New York and London.