Technical method

Latex spheres: A new use in transmission electron microscopy

M. E. TITFORD¹ *From the Electron Microscopy Laboratory, Pathology Department, Brooke Army Medical Center, Fort Sam, Houston, Texas, USA.*

One of the major problems in transmission electron microscopy for tissue examination is accurately determining the size of various structures. This is especially true if the transmission electron microscope (TEM) does not have built-in 'micron markers'.

In our laboratory the major portion of the work is concerned with renal biopsies, including examination of the glomerular basement membrane and pathological changes in its structure. To serve as a built-in reference marker, grids are immersed in an aqueous solution of latex spheres of 0.48μ ($4800\text{ \AA}$) diameter before viewing. This provides a fairly random and even scatter of precisely sized spheres over the entire grid. Since the spheres are directly on the sections, they are magnified at the same rate as the sections.

Method

1 Tissues are processed, embedded and sectioned in the normal manner and the sections mounted upon uncoated 200 mesh copper grids.

2 Sections are stained in uranyl acetate and lead citrate and permitted to dry for five minutes.

3 The solution of latex spheres is gently mixed

by agitation; and a grid, gently held with fine forceps, is dipped in and removed.

4 Excess fluid is removed from the underside of the grid (matte side) with filter paper, and the grid left to dry in a petri dish for five minutes.

5 The grid can then be examined with the transmission electron microscope.

The spheres in solution can be purchased from Ernest F. Fullman, Inc., P.O. Box 444, Schenectady, New York 12301, USA. They are available in three sizes: 0.109μ ($1090\text{ \AA}$) standard deviation 0.0027μ ($27\text{ \AA}$), 0.312μ ($3120\text{ \AA}$) standard deviation 0.0022μ ($22\text{ \AA}$), and 0.48μ ($4800\text{ \AA}$) standard deviation 0.0016μ ($16\text{ \AA}$).

Results

Typically, for one renal biopsy, five grids are prepared containing sections of glomeruli from five separate blocks. Three of these grids are then coated with latex spheres. While examining the grids the pathologist can compare the 0.48μ spheres with the thickness of the basement membrane (normally not exceeding 0.35μ), endothelial cytoplasm fenestrations (usually 0.1μ wide), or the relative sizes of deposits occurring in small nodules or clumps or spread out along the basement membrane. Also, with a little experience, any errors in magnification can be easily detected and corrected in the final prints.

These coated grids can also be used for the modulation stage of high voltage alignment replacing the familiar Holey grid; however, the three-dimensional structure of the sphere prevents their use for final correction of astigmatism.

By this technique, any pathologist unfamiliar with tissue structure at high magnifications can relate the size of various organelles to 0.48μ latex spheres.

¹Communications may be addressed to the author at Ultrastructure Laboratory, Clinical Laboratory, Loma Linda University Medical Centre, Loma Linda, California 92354, USA.

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Fig The thickness of the basement membrane (arrow) in membranoproliferative glomerulonephritis can be compared with the three 0.48μ latex spheres in the adjacent area. $\times 3800$.