IN VITRO SELECTION OF THE MITOTIC CELL FOR SUBSEQUENT ELECTRON MICROSCOPY

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A problem of considerable magnitude in electron microscope technology is that of sampling. This is especially true in the study of mitosis. Even in cell culture, localization of the various mitotic phases by conventional techniques of large scale screening is both time consuming and, in many instances, unsuccessful. In order to circumvent this problem, Bloom has constructed a relatively elaborate and carefully machined marking device. With this device it is possible to select a single cell in the living state and prepare it for ultimate observation in the electron microscope (1, 2). However, the method suffers from a serious drawback, namely, the need for a separate device for each cell if more than one cell is studied at a time. We have found that the same results may be obtained without any auxilliary equipment other than the commercial Leitz diamond-tipped slide marker and the equipment usually found in the electron microscope laboratory. Because of the simplicity of the technique described herein, it is routine to embed 20 or 30 selected cells per day for subsequent electron microscopy.

MATERIALS AND METHODS

Three inch by 1 inch microscope slides are cleaned in a 1:5 nitric acid/sulfuric acid solution. A heavy coat of carbon is then evaporated onto them in the conventional vacuum evaporator (Kinney SC3). The exact thickness of the carbon coat is not critical, but it should be heavy enough to be clearly visible; about $\frac{3}{16}$ inch of a $\frac{1}{16}$ inch diameter carbon rod is the usual amount of carbon deposited. The coated slides are placed in a drying oven at 180°C for 24 hours to insure sterilization and, more importantly, "stabilization" of the carbon film. It was found that, unless this latter step was carried out, the carbon peeled off upon being exposed to liquid medium. A $\frac{1}{8}$ inch deep well is formed on the slides by sealing a stainless

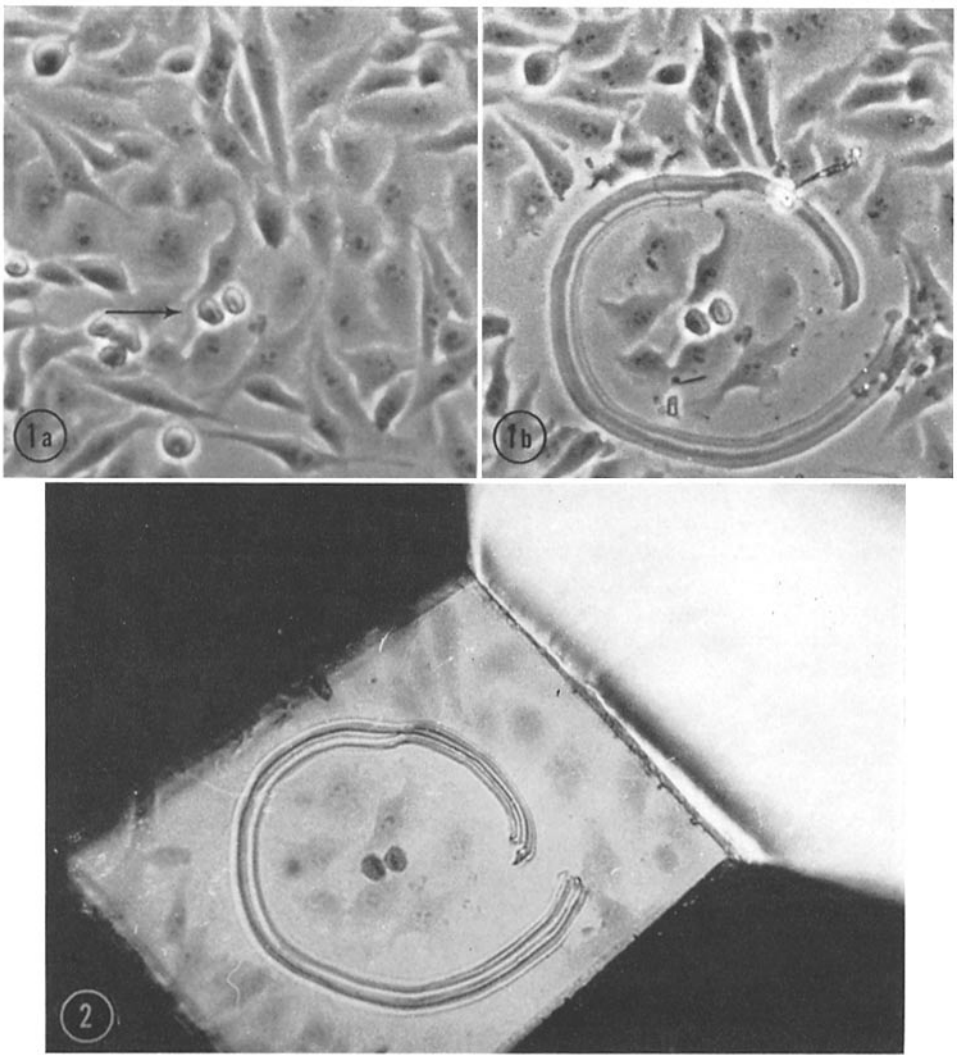

FIGURE 1 *a* Selected HeLa cell (arrow) in telophase. Cell was fixed in glutaraldehyde and OsO_4 according to a previously described schedule (3), and carried as far as 70 per cent Araldite before selection. Phase contrast. × 160.

FIGURE 1 *b* Same cell along with a few neighboring cells after circling with the Leitz diamond slide-marker. Note that the marker has disrupted the carbon coat around the cells. Phase contrast. × 160.

FIGURE 2 Same cells as in Fig. 1, embedded in Araldite. The hardened block has been trimmed to within a short distance of the circular break in the carbon. The cells would ordinarily appear as the mirror image of those shown in Fig. 1 because of the reversed position of carbon coat and cells in this figure compared to Fig. 1; however, for maintenance of the proper orientation, the negative has been reversed and the picture therefore is unchanged. × 160.

steel or glass ring onto them with Dow-Corning silicon grease. The well is then charged with a suspension of HeLa cells which have been detached and dispersed with 0.025 per cent trypsin. Cell density is kept relatively low (about 1.5×10^5 cells/ml) to assure minimal overlap. Attachment and spreading of the cells are carried out for 12 hours at 37°C in an atmosphere of 5 per cent CO_2,

95 per cent air, and 100 per cent humidity. At the appropriate time the medium is removed from the well and replaced with 5.5 per cent glutaraldehyde in isotonic Tyrode's solution (3). Unlike osmium tetroxide in the usual vehicle (4), this fixative preserves mitotic cells with a minimum of obvious structural change, and allows their recognition without ambiguity. After 5 minutes, all but a thin layer of fixative is removed and the slide is placed on a microscope equipped with a Leitz diamond slide-marker and an ocular reticule (Kramer Scientific Co., New York).[1] With a 20 × phase-contrast objective and 12.5 × or 16 × oculars, a cell is selected for circling. Previous calibration with the reticule allows positioning of the cell so that it will be localized in the center of the circle subsequently made with the slide marker (Figs. 1 *a* and 1 *b*). During the process of encirclement, the carbon coat surrounding the selected cell is broken (Fig 1 *b*). The cell is then postfixed in OsO_4, dehydrated, and embedded in the usual way. When the cell is in the pure monomer, a gelatin capsule containing monomer plus accelerator is inverted onto the area of the slide where the encircled cell is located. All excess monomer is carefully wiped away from the periphery of the capsule and polymerization is carried out at 70°C. After 72 hours the capsule is pulled off the slide. Cooling, the use of dry ice, or other means is not necessary since the carbon coat intervening between the glass and the embedding medium easily peels off with the latter if the above precaution of cleaning the periphery of the capsule is followed. Since the carbon coat peels off with the selected cell, examination of the block face under the microscope reveals a clearly delineated circular break in this coat (Fig. 2). Because the cell is in the center of the circle, it is a simple matter to trim the block to a point as close to the cell as the steady hand permits. In practice, we have only approached the outline of the circle since the cell of interest is the only one in mitosis and is thus readily distinguished from its 3 or 4 neighbors when the sections are viewed in the electron microscope. Thin sections are cut with a diamond knife because of its absolutely straight edge. The flatness of the block surface and careful alignment of the microtome chuck permit complete facing of the block within 10 sections. These are then picked up on 100-mesh, Formvar-coated grids and stained for microscopy. Approximately 100 sections may be cut through the mitotic cell, and therefore considerable room is left for missed or poor sections.

[1] It sometimes may be desirable to proceed with the preparation as far as 70 per cent embedding monomer, in order to avoid drying of the thin overlying layer of fixative, allowing more time for cell selection.

RESULTS

An example of the application of the technique is shown in Figs. 3 *a* and 3 *b*. The telophase cell illustrated in Fig. 2 has been followed and photographed at two magnifications. The higher magnification depicts the details of the remaining bridge between the two daughter cells.

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FIGURE 3 *a* Same telophase cell (as in Figs. 1 and 2) as seen in the electron microscope. × 3000.

FIGURE 3 *b* Same telophase cell (as in Fig. 3 *a*) showing details of the intercellular bridge. Numerous spindle fibers and the osmiophilic midbody are seen to advantage. × 33,000.