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TECHNICAL NOTE

A Polycarbonate Filter Technique for Collection of Sorted Cells[1]

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We describe the application of polycarbonate filters to the collection of cells sorted by flow cytometry. Cells are sorted directly onto polycarbonate filters and transferred to microscope slides which are then processed to dissolve the filter and remove pore outlines. The technique results in cell preparations which retain cellular morphology and have high cell recovery of 82-100%.

Key terms: Cell sorting, polycarbonate filters

Visual analysis of cells sorted directly onto microscope slides by a fluorescence-activated cell sorter (FACS) is often hampered by poor cellular morphology and cell recovery. The presence of salt in the sheath fluid of the FACS and cell deformation and lysis occurring during fixation and/or air drying are believed to contribute to these difficulties. We describe the use of polycarbonate filters, often used in diagnostic cytology, in the collection of electronically sorted cells from the sorter. The cells are sorted directly onto the filter and transferred to a microscope slide, which is processed to dissolve the filter and remove pore outlines. In this study we prepared single-cell suspensions from murine solid tumors and small intestine and evaluated the polycarbonate filter technique with respect to cell recovery and morphology after cell sorting.

Materials and Methods

Single cell suspensions of the small intestine were obtained from C3H/He mice (Jackson Laboratories, Bar Harbor, ME) using a modified procedure of Weiser (7), and from the solid KHT sarcoma using neutral protease (5). Tumor and intestinal epithelial cells were fixed in 70% ethanol before staining with chromomycin or propidium iodide, respectively. The fluorescently stained suspensions were analyzed and sorted using a modified FACS II (Becton Dickenson FACS Division, Sunnyvale, CA). During sorting, the fluorescence of chromomycin stained cells was excited at 458 nm (0.2 W) and measured through a Corning 3-71 (Corning Glass Works, Corning, NY) spectral filter. The fluorescence in propidium idiode stained cells was excited at 488 nm (1.0 W) and measured through a Corning 3-69 spectral filter.

For cell sorting, a 5.0-μm polycarbonate filter (13.0 mm, Nuclepore Corp., Pleasanton, CA) was placed in the well of a filter apparatus (6) containing approximately 1.0 ml saline and the apparatus was positioned under the sorting stream. A specified number of cells (e.g., 10,000) was sorted directly into the well and suctioned gently onto the filter using a vacuum line attached to the apparatus. The filters were then washed with 95% ethanol and transferred with forceps to 24-well multiwell plates (Falcon Labware, Division Becton Dickinson, Oxnard, CA) filled with 95% ethanol. As desired, these filters were either stored in a wet chamber or refrigerator until ready for further processing or processed immediately. It was important that the filters remain wet at all times before transfer to glass slides.

Polycarbonate filters were transferred to glass slides, and processed in a manner similar to that described by Gill (2). Briefly, to transfer cells, the filter was placed with the cell side down on a glass microscope slide, covered with a double sheet of filter paper, and gently rubbed several times. The filter was dissolved by placing 2-3 drops of chloroform on it and then immersing the slide in a container of chloroform where dissolution was completed within 30–60 min. The slide was then removed and the cells were fixed in 95% ethanol and air-dried before staining (Wright's or Giemsa) for evaluation of cell morphology and cell recovery.

Figure 1 (*upper*) shows intestinal epithelial cell populations that were sorted onto glass microscope slides, air dried, and treated with 95% ethanol to remove salt crystals. These air dried preparations were characterized by cell lysis, swollen nuclei, and cytoplasmic debris. The same epithelial cell population was sorted onto nuclepore membranes and processed as described above. A photomicrograph of these cells is shown in Figure 1 (*lower*). It is apparent that cells sorted onto polycarbonate filters maintained acceptable morphology including distinct cellular boundaries and nuclear and cytoplasmic regions, and retention of the hemiellipsoid villus cell shape.

We quantitated recovery of sorted cells by depositing a known number of either diploid cells or the larger tumor cells onto the filters, processing as described, staining with Giemsa, and then scoring total

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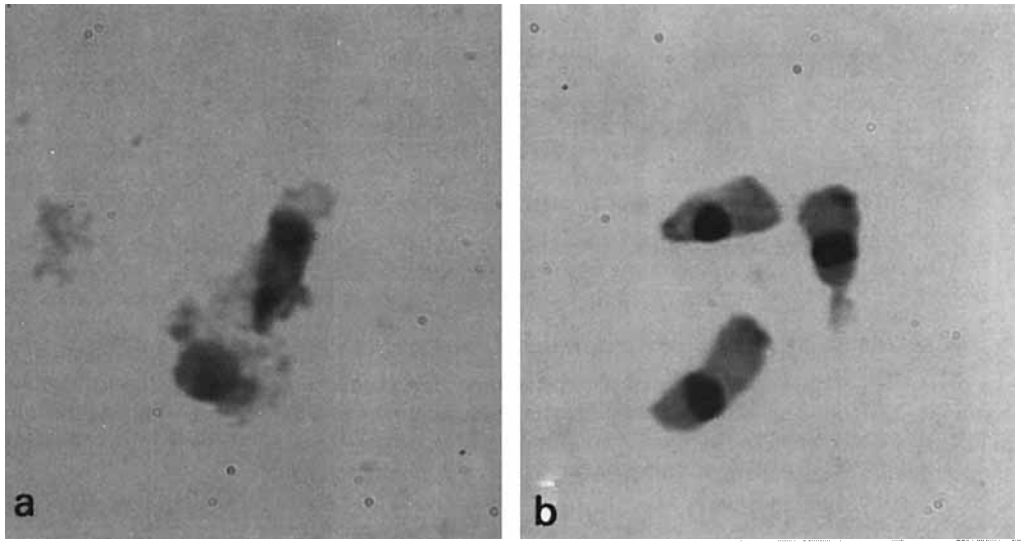

FIG. 1. Photomicrograph of intestinal epithelial cells sorted onto glass microscope slides and allowed to air dry (*upper*) and sorted onto nucleopore membranes (*lower*). Both cell preparations were stained with Wright's stain (magnification: ×800).

Table 1
Cell Recovery

Description	Cells Sorted	% Recovery[a]
Diploid cells	100	82 (75–96)
	500	83 (79–86)
Tetraploid cells	100	96 (81–107)
	500	100 (95–105)

[a] Percentage of recovery is defined as the recorded number of cells sorted per slide divided by the actual number of cells recovered, × 100. Four slides per group were scored, and the values represent the mean percent recovered and the percentage range of the recovered counted cells.

cells per filter area by microscopy. Since the fluorescent DNA-specific stains were retained throughout the procedure, both fluorescent and light microscopy were used for cell counts. Cell recovery data are shown in Table 1. Recovery values ranged between 82 and 100% for the diploid cells and between 96 and 100% for tumor cells with an overall recovery value of 94%. By comparison, the recovery of cells sorted directly onto glass slides is less than 50%, as observed by us (data not shown), and others (4).

Discussion

The polycarbonate filter technique enabled us to obtain microscopic preparations of sorted cells with retention of cellular morphology and high cell recovery (82–100%). We used this procedure to collect sorted cells from both solid tumors and small intestine and good results were obtained with both tissues. High cell recovery with the maintenance of cell morphology allowed microscopic discrimination and identification of sorted cell populations.

Several techniques have been described for collecting and processing cells for subsequent microscopic analysis. These include Leif buckets (3), cytocentrifugation (1), and more recently, a colloidon overlay procedure (4). Any of these techniques may be used for cell recovery; however, the polycarbonate filter procedure has proven the most useful for our studies. Generally, in our hands, Leif bucket preparations have high cell recovery and good cell morphology, but samples can be lost when slides break during centrifugation. In addition, the Leif bucket apparatus is expensive. Cytocentrifuge preparations retain cell morphology but at the expense of cell recovery. The colloidon overlay procedure is reported to give high cell recovery and good cell morphology. However, the technique was described for use with glutaraldehyde-fixed cells, which tend to have better morphology retention than cells fixed with 70% ethanol, the more common flow cytometric fixative.

The polycarbonate filter technique is an easy, rapid procedure to obtain microscopic preparations of electronically sorted cells. Overall retention of approximately 94% of the cells, with maintenance of cellular morphology, reduces sorting time and facilitates the subsequent cytologic analysis of sorted cells.

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