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TECHNICAL NOTE

Preservation of Cells Sorted Individually Onto Microscope Slides With a Fluorescence-Activated Cell Sorter

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Fluorescence-activated cell sorters permit analyses and separation of cell populations based on light scatter and surface immunofluorescence parameters. Since a sorter can deposit individually identifiable cells onto a microscope slide, it was considered of interest to combine the flow measurements with analyses available on cells adhering to a surface as in, for example, morphological studies, cytoplasmic immunofluorescent staining, and mRNA in situ hybridization. A necessary condition for these studies is the preservation of cell structures after sorting. We report here a procedure suitable for this purpose. The most important features of this procedure are A) reducing the saline content of the sorter sheath fluid to about 0.0015 M (one-hundredth that of normal saline) to prevent cell damage due to hypertonicity during drying, and B) coating the substrate with a thin layer of newborn calf serum to promote the adherence of the cells to the substrate during subsequent fixing and staining.

Key terms: Cell sorting, cell preservation, flow cytometry

Stovel and Sweet (1) have described a system for use with a fluorescence-activated cell sorter that allows the sorting of individual particles to separate locations on a substrate such as a glass microscope slide. The present study was undertaken to develop techniques for preserving leucocytes selected and sorted by a cell sorter onto microscope slides in a condition suitable for microscopy, using as a criterion of success comparison with the state of morphological preservation of conventionally stained blood smears or cytocentrifuged splenocytes.

The fluid typically used in flow sorting is normal (0.9% = 0.15 M NaCl) saline or a buffered version of this. An individual sorter-generated drop of normal saline deposited onto an untreated glass microscope slide at room temperature (20°C) and 50% humidity will dry in a few seconds and leave a microscopic area of salt crystals on the slide. A cell in such a droplet will be severely distorted by this drying process, making subsequent microscopic examination of the cell impossible. Furthermore, cells deposited in saline drops on an untreated slide will be washed away during conventional staining procedures.

MATERIALS AND METHODS

Instrumentation

The cell-sorting machine used in the present study was a highly modified instrument based on FACS-II electronics and FACS-IV optical bench (Becton Dickinson FACS Division, Sunnyvale, CA). Three modifications are of relevance to this paper: 1) circuitry enabling the sorting of cells one at a time on demand (such circuitry is now generally available on commercial machines), 2) a simple "phase gate" circuit, which allows the sorting of only those cells that arrive at the detection region in a particular phase relationship with respect to the waveform being applied to the drop drive transducer, and 3) a positioning system to position a microscope slide in the sorting area of the cell sorter. The second and third items were made in our laboratory and are not available commercially. However they are not essential to the techniques described in this paper. Figure 1 is a schematic diagram of the sorting area and positioning device.

The phase gate enhances the percentage of cells that can be recovered when sorting one drop per cell (to effectively 100%) and enhances the drop placement accuracy, but percentages over 90% can be recovered without the phase gate, and placement accuracy is sufficient without phase gating as long as the nozzle diameter is sufficiently large relative to the cell diameter.

The positioning system is a microprocessor-controlled stepping motor driven two-axis state that is essentially the same as that previously described (1).

Other modifications of the sorting equipment described by Stovel and Sweet (1) such as shortened deflec-

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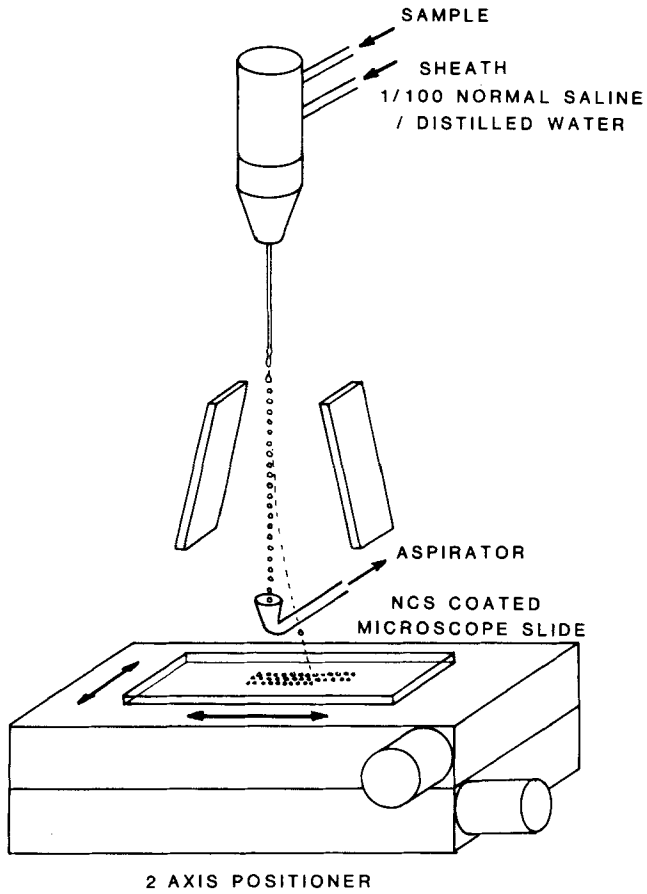

FIG. 1. Schematic diagram of the sorting area and slide positioning system used in this study. NCS, newborn calf serum.

tion geometry and deflection of unwanted drops were found to be unnecessary for this study.

Slide Preparation and Processing

Fifty microliters of newborn calf serum (NCS) (Irvine Scientific, Santa Ana, CA) were spread evenly on the surface of cleaned microscopes slides using a cotton-tipped swab (Chesebrough-Ponds Inc., Greenwich, CN). Dried slides were used for the subsequent sortings. After the sorting the slides were stained with Hemacolor (Harleco, Gibbstown, NJ) or Wright-Giemsa with equivalent results (data not shown). In the Hemacolor staining the slides were fixed 10 sec in solution 1, stained 10 sec in solution 2 and 5 sec in solution 3 and subsequently washed in distilled water.

Procedure

Splenocytes were prepared by mincing the spleens of 4–6-month-old BAB/25 female mice. The cell suspensions were filtered, washed, and resuspended in deficient RPMI 1640 medium (2) (Irvine Scientific) containing 3% of NCS. Mononuclear cells were selected for sorting using their forward (3 to 15°) and obtuse

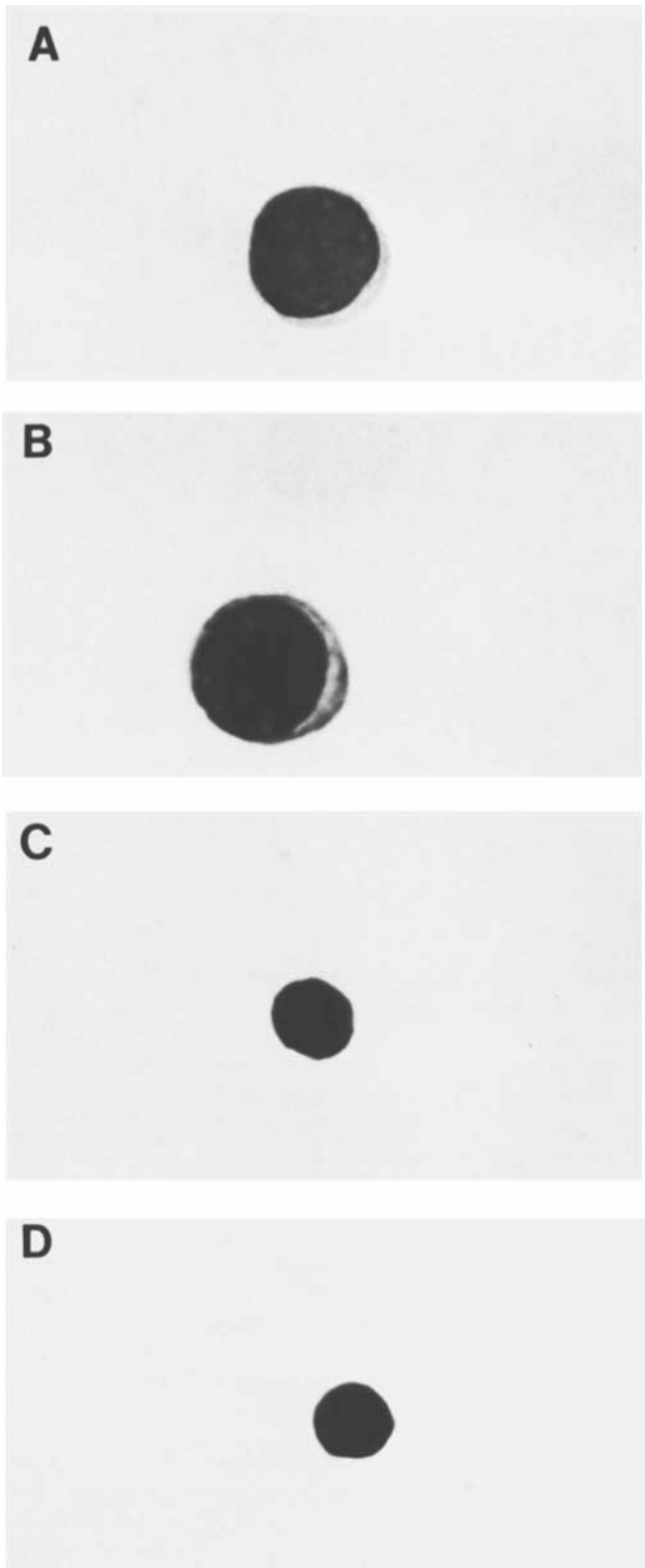

FIG. 2. Morphology of murine spleen lymphocytes deposited by the cell sorter onto NCS-coated microscope slides: diluted saline (A,B) or normal saline (C,D) were used as sorter sheath fluid. Slides were stained with Hemacolor.

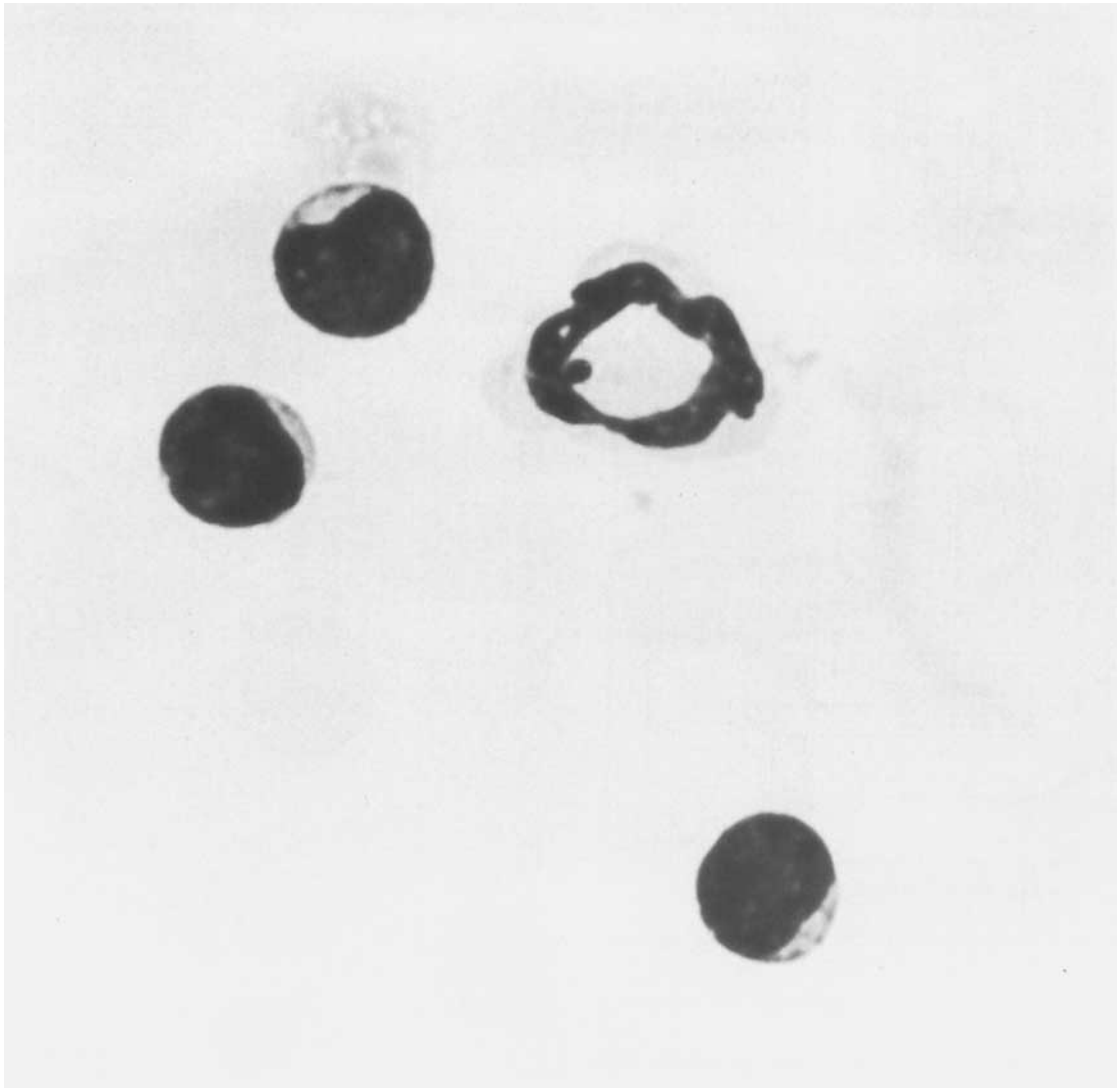

FIG. 3. Morphology of murine splenocyters cytocentrifuged onto an uncoated slide and stained with Hemacolor, presented for comparison with splenocytes sorted and deposited by the cell sorter.

angle (approximately 110 to 150°) light scatter properties. The obtuse angle scatter detection is via an optical fiber mounted next to the standard fluorescence objective and gives information very similar to 90° scatter detection through the objective. The sheath fluid used for sorting was normal saline diluted 1 to 100 in distilled water (0.0015 M NaCl).

RESULTS AND DISCUSSION

Individual cell sorting was performed using the systems described above in (1). Good cell morphology preservation (Fig. 2a,b), as determined by comparison with a control population of splenocytes cytocentrifuged onto an uncoated slide (Fig. 3), was obtained by coating the slides with NCS using 0.0015 M NaCl as sheath fluid on the sorter. This same protocol proved to be suitable for cytoplasmic immunofluorescence studies (K. Hayakawa and R. Hardy, personal communication). The NCS coating was adopted , since the cells used in this study did not adhere well to uncoated slides (data not shown). Good cell retention was also obtained using slides coated with bovine serum albumin or poly-lysine. However, in our hands the latter coatings gave less optimal cell preservation than NCS (data not shown). The dilution of the saline was necessary because physiological osmolarity saline caused the cells to shrink during the drying of the droplets on the slides (Fig. 2c,d). A 1-to-100 dilution of the saline in distilled water was found optimal; higher salt concentrations give less satisfactory results (data not shown). The thickness of the NCS coat was important. Indeed, when cells sere sorted onto slides which were coated by dipping them in NCS, cell shrinkage occurred (data not shown). This shrinkage is likely to be due to the salt from the additional NCS being present

on the slides. The lower salt content of the sheath fluid used in the present studies did not affect the operation or performance of the sorter compared to undiluted saline. We routinely deposited 200 cells per slide with 500 μm spacing between the centers of the droplets. Using this spacing, up to a few thousand cells can be deposited on each slide. Cell yield, i.e., cells actually observed under the microscope, varied between 92 and 96% of the total number of cells sorted onto the slides.

Droplets generated with a 60μm diameter orifice can be deposited with sufficient accuracy that, when the slide is viewed with a microscope using a 40× objective, the row of drops can be followed and the cells located with single axis motion of the microscope stage.

The ability to preserve cell morphology during the sorting of individual cells onto a microscope slide provides an alternative to other methods of slide preparation from sorter-selected cells such as the use of well slides or of a cytocentrifuge. Two or more subpopulations may be deposited in adjacent rows on the slide for easy comparison. Morphology preservation coupled with the ability to match recorded cell sorter data with observable cells on a cell-by-cell basis permits the correlation of observable characteristics with cell sorter measurements, increasing the number of parameters available for each cell.

An additional possible application of this system could be to adapt existing techniques of in situ hybridization (3) to the study of individually sorted cells, thus permitting a molecular biology analysis at the single cell level. Moreover, in addition to the visual microscope process described here, the methods lend themselves well to further automated processing with devices already in use in the image-processing field.

ACKNOWLEDGMENTS

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