Benchmarks

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Standardizing the Counting of Adipocytes in Cell Culture

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Work with cultured cells often requires a precise knowledge of the cell count both to standardize conditions and to carry out quantitative experiments. However, exceptional problems are encountered when working with fat cells (adipocytes). First, the large size of the cells (up to 180 μm in diameter) makes conventional microscopy using glass slides with grids difficult and time-consuming. Second, because the cells contain large amounts of fat, they rapidly float to the surface of the suspension media, making it nearly impossible to contain them in suspension in a pipet long enough to be able to transfer them to a counting chamber. As a result, a uniform distribution of cells in a conventional chamber can seldom be achieved. Conventional automatic cell-counting machines also have difficulty counting adipocytes, because they are unable to reliably distinguish between similar-sized fat cells and fat droplets. Therefore, cumbersome procedures including fixation of cells in osmium tetroxide for 24 h have been used (2). Indirect calculation of adipocyte cell volume and number based on the assessment of the fat content (lipocrit) (1) is also time-consuming, and its accuracy is largely dependent on handling procedures.

We describe a novel method for the rapid assessment of cell number in adipocyte cell cultures using a modified version of an R/S 1000 Sediment Workstation (DiaSys, Waterbury, CT, USA), originally designed for the routine assessment of urine sediments (3).

The heart of the R/S 1000 device is an optical-grade glass flow cell set into an anodized aluminum holder that fits onto, and remains on, the microscope table during the whole procedure. The flow cell is connected by Tygon® tubes (Norton Performance Plastics, Wayne, NJ, USA) to a pump control unit that alternately fills the flow chamber with the cell suspension or with a cleaning solution. Because adipocytes are larger

than most other cells, this instrument is provided with a flow chamber with twice the normal depth and larger input tubing. The flow cell has a constant depth of 250 μm , the visible part of which has a volume of about 10 μL . On the bottom of the flow cell are four sets of 25 grid squares each, of which the smallest square represents a volume of 0.02 μL (Figure 1).

To determine the cell number in a primary culture of human adipocytes, we transferred samples of 250–2000 μL from the agitated culture flask to 2500- μL reaction vials with flat bottoms. Aliquots were diluted by adding culture medium to a total volume of 2000 μL . The cells were then resuspended using a magnetic stirrer. To begin the counting procedure, the aspirator of the R/S 1000 was immersed directly into these suspensions, and the Sample button was pressed. In three seconds, about 220 μL of cell suspension were ingested through the Tygon tubing and into the flow cell. (The flow time, and consequently the amount of the cell-containing fluid ingested, can be altered to suit the user.) The cell suspension is automatically aspirated just past the flow cell but is entirely unable to get near the pump system. The cells were then counted across four grids through the microscope. Because the adipocytes are nearer to the top of the chamber, we used the flow cell with the grids on the upper side (upside-down). Counting the adipocytes at different dilutions, we found a coefficient of variation of 5%–20%, depending on the homogeneity of the suspension (Figure 2). After counting, the Purge button flushes 720 μL of saline through the flow cell within five seconds, thereby cleaning the flow cell and tubing. We have routinely

Figure 1. Human adipocytes (1.6×10^5 cells/mL) seen across two of the grids on the flow cell of the R/S 1000 (magnified 40 $\times$).

Benchmarks

Figure 2. Dilution series of cultured human adipocytes (3×10^5 cells/mL). Each symbol represents a separate reading across one grid (0.5 μ L) consisting of 25 squares.

achieved a throughput of 20–25 samples per hour with this technique.

Our experience shows that cell counts across four grids have relatively small variations resulting in an acceptable standardization of the cell count. Apparently, the cells remain suspended during aspiration and while in the chamber (as opposed to the cell separation that occurs when using a pipet and normal glass slide). Reproducibility depends primarily on the method used for standardizing the cell suspension by magnetic stirring when taking aliquots and during aspiration. In our hands, counting the cells across four grids three to five times was sufficient to obtain reliable estimates of the number of adipocytes in a suspension. Because the ingress and outflow through the aspirator take only seconds, the time required for repeated measurements is minimal compared to that required for a conventional pipet and slide protocol. The automatic operation of the flow cell substantially reduces the duration of the test. Cleaning the apparatus is simple, and aspiration of a second sample before cleaning the chamber is prevented by inactivation of the Sample button until the Purge button is operated at least once.

In summary, the R/S 1000 can, with slight modifications, be used for counting cultured adipocytes. The apparatus is simple to use, and the results, especially with regard to adipocytes, are sufficiently reproducible for most applications. This method can easily be adapted for examining other types of cells. In addition to the basic cell count, the R/S 1000 can also be used for cell sizing or in combination with adequate staining techniques for determining the number of vital cells in a primary culture or the frequency of one type of cell among a mixture of cells. Finally, the thinner version of the flow cell also provides optimum requirements for microscopic work with phase contrast or polarized light.

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