Application Forum

Rapid Analysis of Human Adipose- Derived Stem Cells and 3T3-L1 Differentiation Toward Adipocytes Using the Scepter™ 2.0 Cell Counter

Introduction

Obesity is a prevalent health hazard in many industrialized nations, and its occurrence is closely associated with a number of pathological disorders, such as non-insulin dependent diabetes, hypertension, cancer, and atherosclerosis (1). Because dysregulated adipogenesis and fat cell functions play central roles in these disorders, there has been a surge in efforts to understand the cellular and molecular mechanisms of adipocyte differentiation. Adipocytes are derived from multipotent human mesenchymal stem cells (MSCs), which are found within the bone marrow stroma. MSCs have the potential to differentiate toward many different lineages of mesenchymal tissues including bone, cartilage, muscle, and fat (2).

Human adipose-derived stem cells (ADSCs) are adult stem cells isolated from human lipoaspirates in adipose tissue, and like MSCs, differentiate in vitro toward the osteogenic, adipogenic, myogenic, and chondrogenic lineages when treated with established lineage-specific factors. 3T3-L1 cells are undifferentiated fibroblast-like preadipocytes that are also frequently employed in most adipogenesis studies. These were clonally isolated from Swiss 3T3 cells derived from disaggregated 17- to 19-day-old mouse embryos.

Unique characteristics of adipocyte differentiation include dramatic changes in cell size, shape, membrane potential, metabolic activity, and responsiveness to signals (3). When ADSCs and 3T3-L1 cells commit to the adipocytic lineage, lipid-rich vacuoles accumulate within these cells. We hypothesized that using the Scepter™ cell counter to rapidly assess size distributions of cellular populations would provide a quick, simple method for tracking adipocyte differentiation.

The Scepter™ cell counter (EMD Millipore Cat. No. PHCC20040) captures the ease of automated instrumentation and accuracy of impedance-based counting using the Coulter principle in an affordable, handheld format. The instrumentation has been collapsed into a device the size of a pipette, and uses a combination of analog and digital hardware for sensing, signal processing, data storage, and graphical display in the form of a histogram. The 40 μm - and 60 μm -aperture sensor tips are engineered with a micro-

fabricated, sensing zone that enables discrimination by cell size and cell volume at sub-micron and sub-picoliter resolution, respectively. The histogram output provides a quick snapshot of cell size and density.

This study outlines a method for tracking adipogenic differentiation of ADSCs and 3T3-L1 cells and subsequent sample analysis using the Scepter™ cell counter to measure these changes. We have employed Scepter™ technology for determining cell size and volume, as well as lipid vacuole staining using Oil Red O as a cross-validation histology screen to investigate the relationship between cell differentiation and cell size changes. Although the Scepter™ cell counter was intended primarily as a cell counting device, we demonstrate how this cell counter can also function as a reliable tool to track phenotypic change.

Figure 1. Preadipocytes (ADSCs) can be distinguished from differentiated adipocytes based on cell size. Using a 60 μm sensor, the Scepter™ cell counter enabled the discrimination of cell types based on size, with high resolution. ADSCs were measured at three key time points: Day 0 (control), 7 days after exposure to differentiation conditions, and 14 days differentiated. As indicated by the histogram data, cells gradually increased in size from 15 to 21 μm over the 14-day differentiation.

Figure 2. Oil Red O staining was used to measure the increased lipid content of adipocytes compared to undifferentiated cells. Undifferentiated ADSCs and differentiated samples taken from the same critical time points (7 and 14 days) were stained with Oil Red O. Oil Red O stains lipid vacuoles, enabling visual quantification of lipid content in cells by microscopy (40X objective). As seen, increased lipid content, which is common for mature adipocytes, correlates with the cell size measurements determined by Scepter™ counting.

Materials and Methods

Adipogenesis Differentiation: ADSCs

ADSCs were plated at a density of 50,000 cells per well into gelatin (EMD Millipore Cat. No. ES-006-B)-coated 12-well culture dishes with 1 mL medium per well. Cells were incubated at 37 °C in a 5% CO₂ humidified incubator for 4 days. When the cells were 100% confluent, medium was aspirated from each well and replaced with 1 mL Adipogenesis Induction Medium (EMD Millipore Cat. No. SCR020). This medium change was set as differentiation day 1.

Medium was replaced with fresh Adipogenesis Induction Medium every 2-3 days for 14 days. After 0, 7, and 14 days of differentiation, adipocytes were fixed and the lipid droplets stained with Oil Red O Solution. Lipid droplets could be detected by microscopic examination as early as 5 days into the differentiation period.

Adipogenesis Differentiation: 3T3-L1 cells

3T3-L1 cells (ATCC Cat. No. CL-173TM) were plated in 10% calf serum/DMEM Medium at a density of 50,000 cells per well into 12-well culture dishes with 2 mL medium per well. Cells were incubated at 37 °C in a 10% CO₂ humidified incubator for 4 days.

When the cells were 100% confluent, the medium was aspirated from each well and replaced with 2 mL MDI Induction Medium. This medium change was set as differentiation day 0.

At differentiation day 2, the medium was replaced with Insulin Medium. The medium tended toward greater viscosity as free fatty acids were produced by the cells and secreted into the medium. At differentiation day 4, the medium was replaced with MDI Induction Medium. At differentiation day 6, the medium was replaced with Insulin Medium. At differentiation day 8, the medium was replaced with 10% FBS/DMEM.

Figure 3. Cell diameters of ADSCs and 3T3-L1 cells undergo comparable increases during adipocyte differentiation (adipogenesis) as measured using the Scepter™ cell counter.

Cells were subsequently fed with 10% FBS/DMEM every two days. After differentiation, >90% of cells were mature adipocytes with accumulated fat droplets. After 7 and 14 days of differentiation, adipocytes were fixed and the lipid droplets stained with Oil Red O Solution.

Scepter™ Cell Counting

The Scepter™ cell counter was used to count samples following the detailed on-screen instructions for each step of the counting process. Briefly, the user attaches a 60 µm sensor tip, depresses the plunger, submerges the sensor into the sample, then releases the plunger drawing the cell suspension into the sensor. The Scepter™ cell counter detects each cell passing through the sensor's aperture, calculates cell concentration, and displays a

size-based histogram as a function of cell diameter or volume on its screen. Scepter™ 2.1 software was then used to upload test files from the device and perform subsequent data analysis to determine cell size and relative cell frequencies for the preadipocyte cultures (ADSCs and 3T3-L1 cells). The initiation of differentiation culture conditions was noted to mark day 0, differentiation at day 7, and differentiation at day 14.

Results

Preadipocyte cultures were treated with established lineage-specific factors for 14 days to drive adipogenesis and formation of terminally differentiated adipocytes. Both ADSCs and 3T3-L1 cells were used in this study since both cell types have been commonly utilized when performing in vitro analysis of adipogenesis. Moreover, both cell types have been well characterized and contain the same cellular machinery as seen in multipotent mesenchymal stem cells.

Adipose-Derived Stem Cell Differentiation Analysis

ADSCs were induced to differentiate toward adipocytes. Samples were gently trypsinized and analyzed using the Scepter™ cell counter. Undifferentiated cells exhibited a mean cell diameter of 14.9 µm; cells differentiated for 7 days had a mean cell diameter of 18.4 µm; cells differentiated for 14 days had a mean cell diameter of 21.1 µm (Figures 1 and 3). Concurrently, the same samples from different wells were stained with Oil Red O (which stains lipid vacuoles) and counterstained with hematoxylin. Histology images were captured using a Leica DMI 6000, 40X objective lens.

3T3-L1 Cell Differentiation Analysis

In a similar fashion, 3T3-L1 cells were induced to differentiate toward adipocytes. Samples were gently trypsinized and analyzed using the Scepter™ cell counter. Undifferentiated cells exhibited a mean cell diameter of 15.4 µm; cells differentiated for 7 days had a mean cell diameter of 18.8 µm; and cells differentiated for 14 days had a mean cell diameter of 20.3 µm (Figures 3). Concurrently, the same samples from different wells were stained with Oil Red O and counterstained with hematoxylin (figure 2). Histology images were captured using a Leica DMI 6000, 40X objective lens.

Using the Scepter™ cell counter to determine cell size and distribution of the cell cultures, an increase in cell size relative to the number of days during cell differentiation was accurately determined. Each experiment was performed on three separate occasions to validate our findings, and, each time, cross-validation using Oil Red O staining confirmed

these results. Small standard deviations of the mean measured diameter of three cell samples demonstrated the precision of the Scepter™ cell counter.

Both ADSCs and 3T3-L1 cells were performed in triplicate assays (three independent experiments, plus three samples per experiment) and standard deviation values were determined. These data demonstrated the precision of the Scepter™ cell counter for performing cell size determination across many test samples.

Conclusions

Mesenchymal stem cell research and understanding its relationship to adipogenesis remains an important area for research with the realization that society may be in the early stages of a global wave of obesity. The ability to isolate, expand, and direct the in vitro differentiation of ADSCs and 3T3-L1 cells to particular lineages provides the opportunity to study events associated with commitment and differentiation, and, in the case of this study, the development of terminally differentiated adipocytes.

The Scepter™ cell counter provides a rapid, easy, and inexpensive method for assessing ADSC and 3T3-L1 differentiation in culture. This handheld, automated cell counter delivers precise, reliable cell size measurements which can provide the researcher a quick snapshot of the differentiation status and the success of their differentiations in culture. As a result, researchers can fine-tune culture conditions and adjust analysis parameters to maximize information obtained from each experiment, make better experimental decisions, and ultimately, enjoy more productive research.

Related Products

Description	Qty/Pk	Catalogue No.
Scepter™ 2.0 Handheld Automated Cell Counter		
with 40 µm Scepter™ Sensors (50 Pack)	1	PHCC20040
with 60 µm Scepter™ Sensors (50 Pack)	1	PHCC20060

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