Application Note

Cell Analysis Module

Measurement of mesenchymal stem cell numbers using "MSC Count"

＜BioStudio-T usage example＞

When cell growth is evaluated over time for the purpose of selecting a batch of serum, developing culture conditions, or examining the course of the effects of drugs over time, cell numbers are periodically counted. Cells seeded in multi-well plates are dissociated and cell numbers counted at the same time every day for several days. A cell growth curve is generated from the counted results to confirm cell proliferation. Such processes are time-consuming and labor-intensive work, are difficult to standardize, require skillful techniques, and consume valuable resources including large numbers of cells, serums and media. Furthermore, even if using the cell suspension that has been adjusted by counting the number of cells contained, an error in the actual seeded number of cells is unavoidable.
By using the NIS-Elements image analysis software in combination with the Cell Analysis Module "MSC Count", it is possible to measure the number of cells from phase contrast images of mesenchymal stem cells (MSCs) in culture.
The plate in which MSCs were seeded was placed in BioStudio-T in a CO_2 incubator for several days and images of the cells were captured over time. Cell numbers were counted from phase contrast images using the Cell Analysis Module.

Observation device

- BioStudio-T (BS-T04A) (Nikon, MLA20000)

Image analysis software

- NIS-Elements AR ver. 5.30.02 (Nikon, MQS31000)
- NIS-A General Analysis (Nikon, MQS43110)
- NIS-A Upgrade to GA3 (Nikon, MQS43150)

Cell Analysis Module

- PC-CO-01 MSC Count (Nikon, MQS60000)

Cells

- Bone marrow-derived mesenchymal stem cells hTERT, HPV E7-introduced, immortalized cell line, UE7T-13 (JCRB1154, JCRB cell bank)(1)

Reagents and materials

- Gibco™ DMEM, low glucose, pyruvate (Thermo Fisher Scientific, 11885084; with Low Glucose, L-Glutamine, Sodium Pyruvate, without HEPES)
- Gibco™ Fetal Bovine Serum, mesenchymal stem cell-qualified, USDA-approved regions (Thermo Fisher Scientific, 12662-029)
- Gibco™ PBS (-), pH 7.4 (Thermo Fisher Scientific, 10010023)
- TrypLE™ Select Enzyme (1X), no phenol red (Thermo Fisher Scientific, 12563011)
- StemSure® 0.1w/v% gelatin solution (Fujifilm Wako Pure Chemical Industries, 190-15805)
- Costar® 6-well Clear TC-treated Multiple Well Plates (Corning, 3516)

Methods

UE7T-13 cells were seeded in each well of a 6-well plate at cell densities of 1.0 x 10^4, 2.0 x 10^4, and 4.0 x 10^4 cells per well. BioStudio-T (4X model) was set in a CO_2 incubator. The cells were cultured at 37°C, in a humidified atmosphere containing 5% CO_2. 9 x 12 image fields at the center of the well were captured with phase contrast. The values of each Z-position that was manually adjusted at the center of each well were set in the grid menu, and time-lapse images were captured with the image AF setting. The captured tiled images were imported into NIS-Elements as time-lapse images, and image fields (approx. 16 mm x 14 mm), which were not affected by meniscus, were cut out from the captured images. They were then analyzed using the Cell Analysis Module "MSC Count" and the number of cells were counted. A growth curve was created from the obtained values to confirm cell growth.

Results

The image fields (approx. 16 mm x 14 mm) not affected by meniscus were cut out from the original phase contrast images after cells were cultured for six days, and analyzed using the Cell Analysis Module "MSC Count". The cell number was automatically counted by NIS-Elements. A representative image and analyzed image are shown in Fig. 1 and growth curves were created from the analyzed result (Fig. 2).

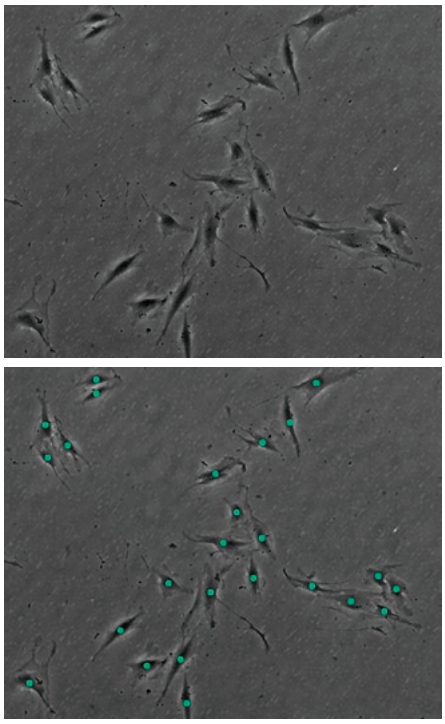

Figure 1: Phase contrast image and masked image of UE7T-13 cells

(Above) A part of a phase contrast image taken four hours after seeding UE7T-13 cells at a density of 2.0 x 10^4 cells per well. (Below) Image after application of NIS-Elements' Cell Analysis Module "MSC Count".

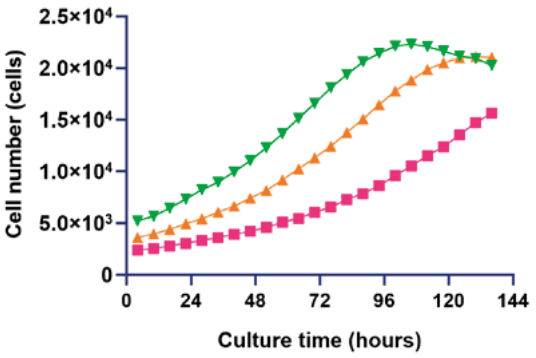

Figure 2: Growth curve of UE7T-13 cells

UE7T-13 cells were seeded at 1.0 x 10^4 (magenta), 2.0 x 10^4 (orange), and 4.0 x 10^4 (green) cells per well. Cell numbers were quantified from image field (approx. 16 mm x 14 mm) not affected by meniscus, after application of Cell Analysis Module "MSC Count".

Summary

- It is possible to quantify UE7T-13 cell numbers from the phase contrast image using NIS-Elements without detaching or dispersing the cells during the cell culturing process.
- The measured values can be output in CSV format, and used for creating graphs with software such as TIBCO Spotfire® and Microsoft Excel®.
- Cells used for evaluating cell growth can be used again in the next experiment, because the "MSC Count" can evaluate the sample in a non-invasive way.
- Since cell numbers can be quantified while the cells are being cultured, the appropriate timing for passage and experiments can be determined using the results.

Reference

1. Taisuke Mori et al., Combination of hTERT and bmi-1, E6, or E7 induces prolongation of the life span of bone marrow stromal cells from an elderly donor without affecting their neurogenic potential, *Mol Cell Biol*., 25(12): 5183-5195, 2005.

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