Cellular and Molecular Biology
ISSN: 1165-158X

An Automated Microscope System to Analyze Cell Proliferation

Ji Eun Kwon, Seungyeon Park, Yeong-Ho Cha and Sun Young Park*

Abstract

Cell proliferation occurs via cell division under appropriate conditions, including a controlled cell cycle and cellular homeostasis, and the abrogation of cell proliferation results in pathological states such as cancer and senescence. Cell-based in vitro experiments were conducted in an optimal cell proliferation environment, and the analysis of cell proliferation is a critical tool for exploring cellular homeostasis and developing drugs to modulate cell growth. Although colorimetric assays are limited by the small culture area and the short tracing period and cell counting using a hemocytometer requires multiple plating for each time point to be analyzed, these two methods have been used widely for the analysis of cell proliferation. In this study, we aimed to develop a robust method to detect cell proliferation via an automated microscopic cell-number analysis (AMCA) system to overcome the limitations of traditional methods. In contrast colorimetric assays showed reduced sensitivity with increased cell density, AMCA showed constantly reliable detection. Also, the AMCA system exhibited a comparable cell proliferation trend with the direct whole-cell counting method. In conclusion, we suggest AMCA as an alternative method to analyzing cell proliferation with expanded spatial and culture period conditions and expect AMCA to provide an e f cient drug developing experiment.

Namricon-ro, Namricon-myeapopGyeapgsan-siopGyeapgsangbuk-do, 38695, South Korea, Tel: 82-53-819-8025; E-mail: sunnypark@clearlab.com

Received: 10-Dec-2021, Manuscript No. CMB-21-49428; **Editor assigned:** 13-Dec-2022, PreQC No. CMB-21-49428(PQ); **Reviewed:** 08-Jan-2022, QC No. CMB-21-49428;

automated cell count system, four sets of cells (2.5 × 103 cells/well) were plated in a 96-well plate and analyzed on Days 1 to 4. e absorbance of formazan was measured a er 60-min substrate incubation.

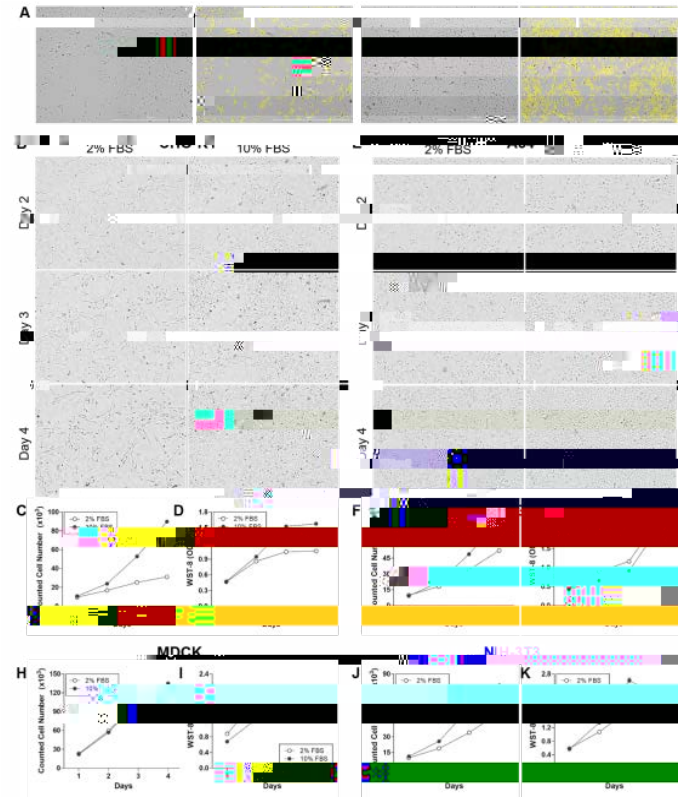

Figure 2: Analysis of cell proliferation via the WST-8 assay and AMCA system. (A) De fning cell boundaries by the phase di ference in CHO-K1 cells for the analysis of cell numbers in a feld in low-density (left) and high-density (right) CHO-K1 cells. (B–E), Vero (E–G), MDCK (H and I), and NIH-3T2 (J and K) cells were plated in 96-well plates (2.5 × 103 cells/well, n = 6) or 6-well plates (5 × 104 cells/well). (B, C, E, F, H, and J) AMCA was conducted with plated cells in a 6-well plate by the detection of the number of cells in 24 randomly selected felds every day for 4 days. (B and E) Representative images of CHO-K1 (A) and Vero (D) (×40) cells on Days 2 to 4. (C, F, H, and J) Cell counts are the sum of the numbers from 24 felds. (D, G, I, and K) Cells plated in 96-well plates were incubated for the indicated number of days, and the absorbance at 450 nm was measured after reaction for 30 min with the WST-8 substrate (n = 6).

AMCA as an analysis method for 96-well-scale cell culture.

Cell counts of the designated elds are su cient to represent the total cell count

To demonstrate whether the sum of cell counts from designated elds was su ciently representative of the total cell count. e cells were plated in 6-well plates and the cell numbers were determined by the AMCA system or counting the number of cells using hemocytometer. As shown in Figure 3C, the total count of Vero cells, determined using a hemocytometer, was higher when cells were grown in medium supplemented with 10% FBS than 2% FBS. Consistent with the total cell counts, AMCA also revealed the higher proliferation rate with 10% FBS supplementation than 2% FBS (Figures 3A and 3B). Further, HeLa (Figures 3D-3F), CHO-K1 (Figures 3G and 3H), and MDCK (Figures 3I and 3J) cells also exhibited greater levels of proliferation when grown in 10% FBS-supplemented medium compared with 2% FBS-supplemented medium measured by both methods. ese results suggest that the assessment of cell growth using AMCA may be able to replace the need for total cell counts.

Discussion

Although the purpose of analyzing cell proliferation varies, a considerable number of studies have used colorimetric assays, such as the MTT, XTT, and WST-8 assays, owing to their accessibility and sensitivity [10]. Colorimetric assays analyze the enzymatic activity of viable cells plated in 96-well plates via the detection of the formazan product. Unlike the MTT assay, which requires a solubilization step, the XTT and WST-8 assays detect the absorbance of the formazan product in live cells. However, owing to the toxicity of the substrate, the period over which proliferation can be tracked is limited to 2 days.

e current study aimed to develop a long-term analysis method for cell proliferation without disturbing cellular homeostasis, and took advantage of an automated microscope system to recognize the cell boundary using phase di erence (Figure 2A). e AMCA system allows the analysis of identical elds for each detection and provides images that indicate the increase in cell-number (Figures 2B, 2E, 3A, and 3D). Culturing cells in 96-well plates results in a higher possibility of inhibiting cell growth owing to the limited area (Figure 2D and 2K). As automated microscopic analysis can be performed on a larger area, such as a 6-well plate or a 100-mm culture dish, the analysis of proliferation over a longer time is possible compared with 96-well plate-based colorimetric assays. us, the advantage of extended tracking of cell proliferation may provide the exible application of the AMCA system in cell culture-based in vitro drug e cacy experiments.

e most straightforward approach to cell proliferation is to count the number of cells a er the collection of all the cells in the culture plate. Traditionally, counting the number of trypan blue-stained cells with hemocytometer is used and automated cell counting instruments have been developed to minimize human labor and maximize accuracy. However, counting all the cells in culture plates require that culture is stopped in the culture plate and multiple plating is needed to trace

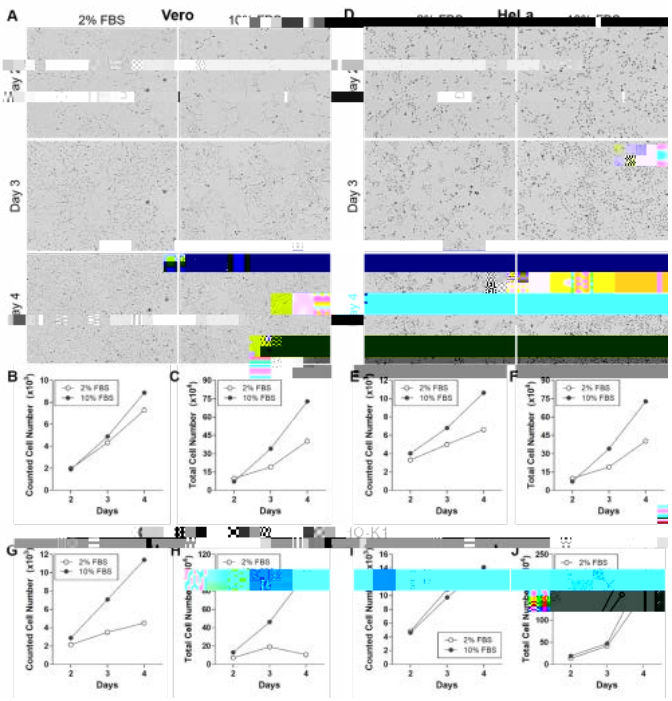

Figure 3: Analysis of cell proliferation via cell counting using a hemocytometer and the AMCA system. Vero (A–C), HeLa (D–F), CHO-K1 (G and H), and MDCK (I and J) cells were plated in 6-well plates (5 × 104 cells/well). (A, B, D, E, G, and I) AMCA was performed on cells plated in a 6-well plate by detecting the cell numbers of 24 randomly designated felds every day for 4 days. (A and D) Representative images of Day 2 to Day 4 of Vero (A) and HeLa (D) (×40). (B, E, G, and I) Cell counts are the sum of the numbers from 24 felds. (C, F, H, and J) Cell numbers on the indicated days were obtained by counting live cells (trypan blue-negative) in each well using a hemocytometer (n = 3).