ORFLO | Personal Cell Analysis

TECH NOTE | 11/2011

Monitoring Cell Culture Health with Moxi Z's MPI

Introduction

Scientists expend significant time, labor and resources on maintaining consistent, healthy cell cultures to support their research efforts. In addition to the initial, significant expenditures for core equipment, there are substantial recurring costs for materials that ensure optimal culture environments including sterilized consumables, media, reagents and growth factors. Beyond material costs, successful cell culture requires proper training and attention to ensure aseptic technique and use of cell-specific growth/treatment protocols. Overall, the motivating force behind all these investments is the importance of maintaining consistent and predictable cell phenotypes and behaviors, often from registered cell lines, for defensible interpretation and analysis of scientific findings.

Common factors that can adversely affect culture health, and consequently phenotype, include microbial contamination, poor culture technique (prolonged protease exposure, over trituration), loss of pH buffering (i.e. low CO2 levels), temperature fluctuations, nutrient deprivation and hypoxia. Experienced cell biologists rely on many indicators to routinely evaluate the state of their culture health including cell morphology, growth rates, live/dead assays and phenol-containing media color. Vigilance and early intervention can help researchers save cultures, avoid spread of contamination, and prevent the inclusion of an unhealthy cell phenotype in research.

The Moxi Population Index (MPI) provides a novel approach for monitoring the health of cell cultures. Similar to visual inspection of morphology and media color/composition, this test is performed automatically with every Moxi Z cell count, without the need for additional reagents. Specifically, MPI provides a rapid quantitative assessment of culture health by generating the ratio of the count of the curve-fit (largest relevant) cell population to that of the entire particle population. Because the shrinking and breaking up of necrotic and apoptotic cells, as well as microbial contamination, contribute to increases in smaller size particle/debris counts, these unhealthy culture conditions are correspondingly reflected in lowered MPI values. Fig 1 shows the effect of culture health on the MPI metric and on the cell size histogram profile, using MoxiChart software provided with the system Overall, the MPI provides critical new insights into culture health in a simple and automated manner. Examples of the use of MPI in assessing microbial infected cultures and CO_2-deprived cultures are shown below.

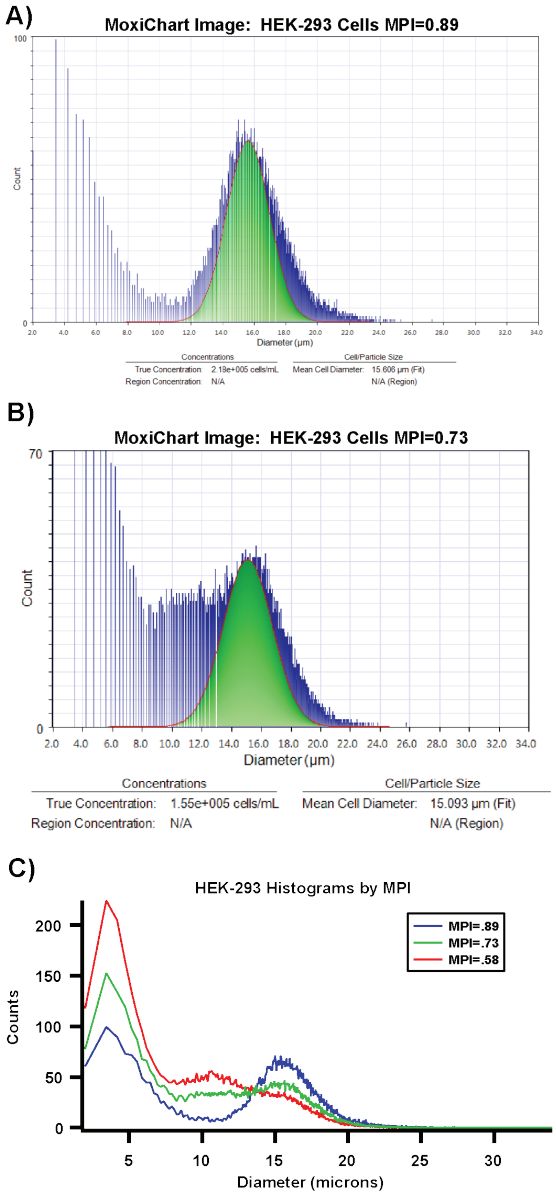

Fig 1 – A) MoxiChart image of a healthy HEK culture (MPI=0.89) B) Moxichart image of a HEK culture spiked with identical passage cells killed through overnight incubation (37° C) in a nutrient-free, sodium fluoride containing diluent. In (B), the shrunken dead are distinguishable as a peak in the 8 – 12 µm region. The lower MPI (0.73) reflects this decreased culture health. C) Superimposed histograms show the effect of decreased viability on the size histogram profile and MPI values.

Detection of Microbial Contamination

One of the most common and disruptive contributors to the demise of a cell culture is microbial contamination. While most common bacteria are below the size detection threshold of Moxi Z, the micro-colony aggregates that they collectively form can be measured at the lower end of the Moxi Z size scale. Fig 2 highlights the effect of microbial contamination on Moxi Z - measured size histograms for pure media with no antibiotic (Fig 2A), with media containing penicillin-streptomycin (Fig 2B) and on an HEK-293 culture. Notably, in the case of the media measurements, measurable increases in particle counts were registered prior to the visualization of media turbidity. This would have a direct impact on suspension cell culture counts, and, as shown in Fig 2C is measurable in both the supernatant as well as post detachment counts of an adherent culture (HEK-293) resulting in an MPI decrease from 0.93 to 0.74.

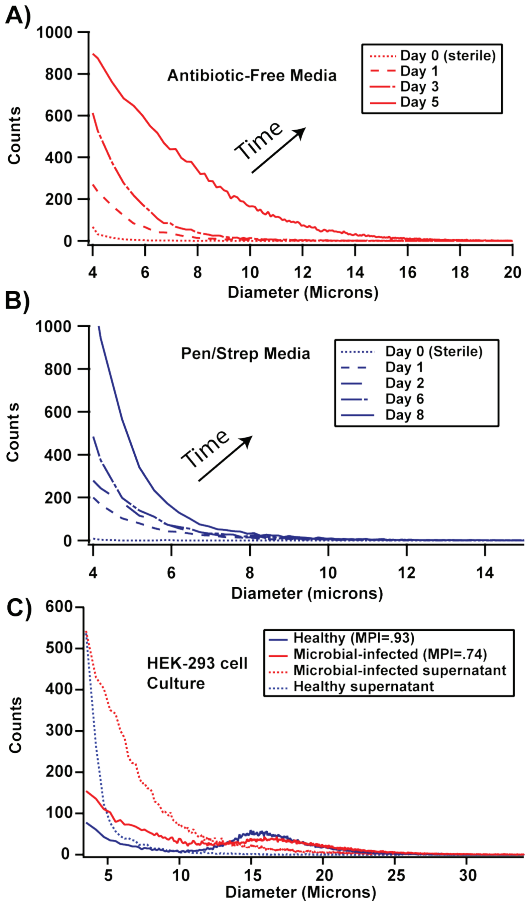

Fig 2 – 20 µL of bacterial infested media was added to 15 mL of MEM containing 10% FBS A) without antibiotic B) with penicillin-streptomycin added. Daily monitoring of cell counts revealed increases in measured microbial counts, even prior to visualization of solution turbidity. C) Similar contamination of a HEK-293 cell culture resulted in a change in the size histogram profiles and decrease in the MPI values from 0.93 for the healthy culture (red solid line) to 0.74 for the contaminated culture (blue solid line) Lines represent the average of three count histograms for each condition

Monitoring CO2 Buffering

Loss of incubator CO_2 supply (commonly held at 5%) eliminates the pH buffering capacity of the universal Sodium Bicarbonate buffer systems. This can occur commonly as lab CO_2 cylinders empty, resulting in pH increases that can disrupt cell health. Fig 3 shows the effect of two days of CO_2 deprivation on both Jurkat E6-1 and CHO-K1 cells as reflected in the Moxi Z size histograms and MPI values. The CO_2 deprived culture (red traces) show clear increases in the dead cell/debris counts relative to the control (5% CO_2) cases. This is quantitatively reflected in the MPI values that decreased from .88 (control) to .66 (CO_2 deprived) for the Jurkat cell culture and from 0.89 (control) to 0.60 for the CHO culture.

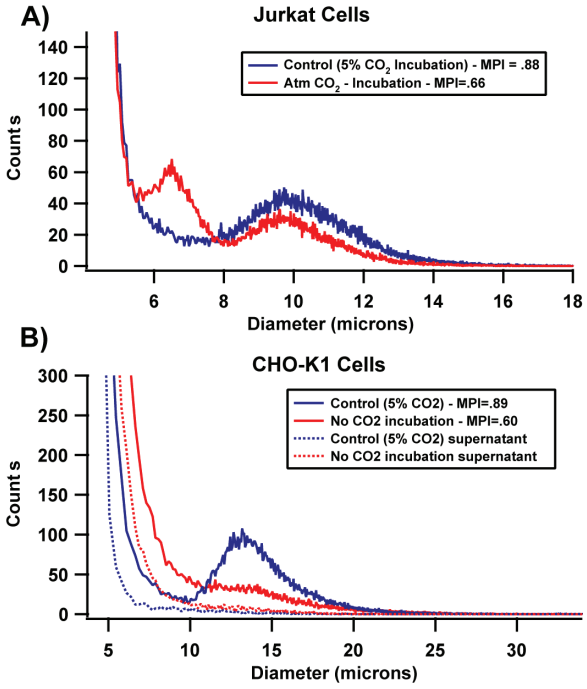

Fig 3 – Effect of loss of CO_2 buffering on A) Jurkat cells and B) CHO-K1 Cells. MPI values drop for both cell types after two days of incubation in atmospheric CO_2 only.