Simplifying Progress

Impact of Pipetting Errors on Reproducibility of Cell-Based Assays

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Abstract

For the successful use of the cell-based system, even distribution of cells, avoidance of bubbles and monitoring of cell growth dynamics are important. Good pipetting techniques and practices help to reduce inter-assay variance and improve reliability of results.

Introduction

Variance within experimental replicates and inter-assay variance can be a significant unreliability factor in cell-based assays. It has been shown that variability during manual processing can cause significant changes in the total viable cell concentration and target phenotype purity during directed differentiation of mouse embryonic stem cells (Veraitch et al 2007), proposing that reducing the variance with best sample preparation practices will provide more reliable and reproducible results.

Materials and methods

Well-to-well variation in cell seeding
A cell suspension of 50,000 HT-1080 cells/mL was titrated and immediately dispensed into two separate reservoirs. For plate 1, the cell suspension was mixed 3 times prior to dispensing 100 µL into each row by gently agitating the reservoir and pipetting 3 times between each row. For plate 2, the cell suspension was not mixed between rows prior to cell seeding. Phase confluence of each well was monitored for 3 days in IncuCyte® live-cell analysis system.

Untreated MDA-MB-231 in 50,000 cells/mL dilution was pipetted to 24 well plate without mixing the suspension prior or between pipettings. The phase confluence of each well was monitored for 4 days in IncuCyte® live-cell analysis system.

Determining HeLa cell viability with formazan based assay
In this experiment HeLa cells grown in 10% FBS DMEM were counted and a gradient of cell densities from 5,000 to 25,000 cells was prepared from the stock in 96-wells with either mixing gently by pipetting up-and-down between steps or without mixing. The number of live cells seeded into each well was measured by absorbance using a cell viability assay (formazan based, cell counting kit-8, Sigma Aldrich).

Effect of bubbles in cell viability assay results
HeLa cells grown in 10% FBS DMEM were counted and a gradient of cell densities from 5,000 to 25,000 cells per well was prepared from the stock in 96-wells either by using forward (basic) pipetting technique with blow-out in the end or by using reverse pipetting. The number of live cells seeded into each well was measured by absorbance using a cell viability assay (formazan based, cell counting kit-8, Sigma Aldrich).

Results

Seeding of cells is an important component of the assay. Poor mixing is one of the main factors leading to variance in cell seeding and the effect of initial cell count can be seen in growth rate throughout the entire experiment. Formation of bubbles lead to uneven distribution of cells and increase deviation.

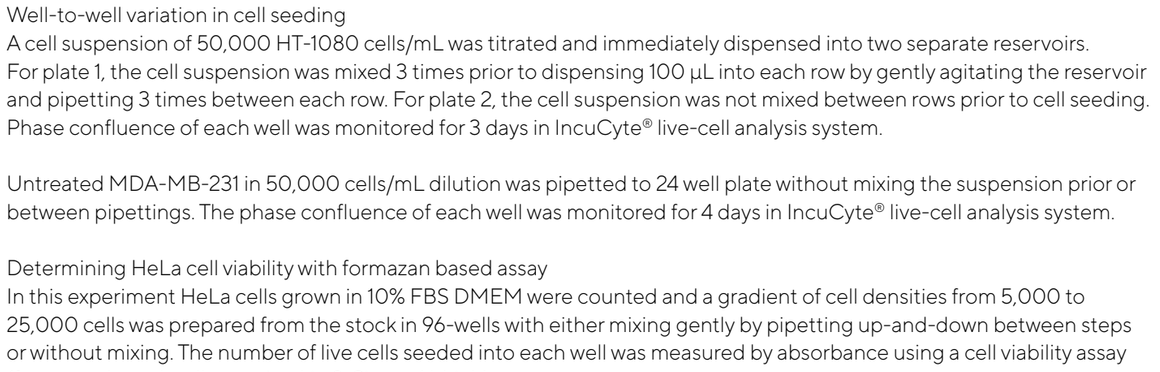

Figure 1: Well-to-well variation in cell seeding Graphs illustrate a greater variation in initial cell seeding density for Plate 2 (Figure 1 B), where HT-1080 cells were not mixed prior to cell seeding in comparison to Plate 1 (Figure 1 A) where cells were mixed pipetting up-and-down between each row. The area under the curve of confluence over time for each well was used to calculate the coefficient of variation for each plate (Plate 1 CV = 4.67%, Plate 2 CV = 12.57%).

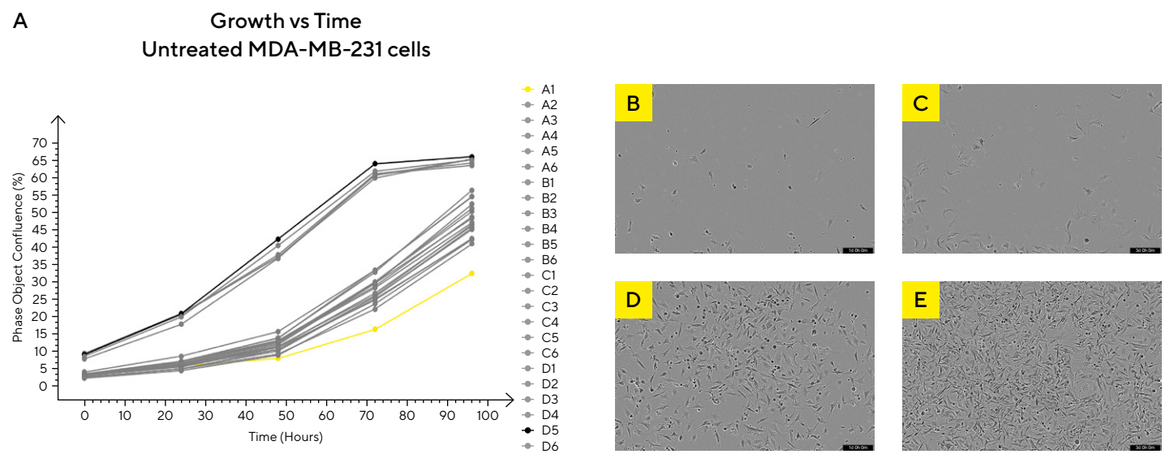

Figure 2: Well-to-well variation in cell seeding The growth curves of untreated MDA-MB-231 cells (A) show that inadequate mixing between pipettings lead to variance in cell counts and confluence. The higher initial cell number in well D6 (image C) in comparison to A1 (image B) lead to different growth rate and confluence and the deviation is still significant even after 72 hours of cultivation (A1 image D and D6 image E)

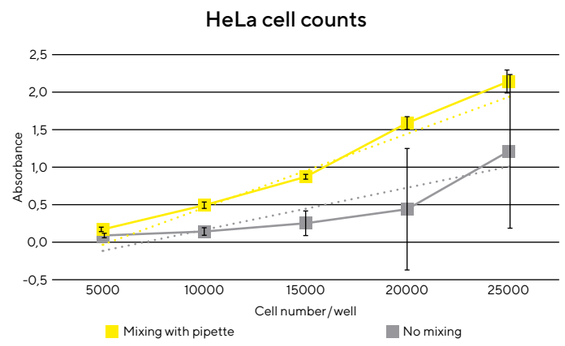

Figure 3: Determining HeLa cell viability with formazan based assay. When a gradient of cell densities on a 96-well plate is done by mixing in each step with the pipette, the deviation between replicates is smaller and the cell count linear in comparison to when the cells were seeded without adequate mixing.

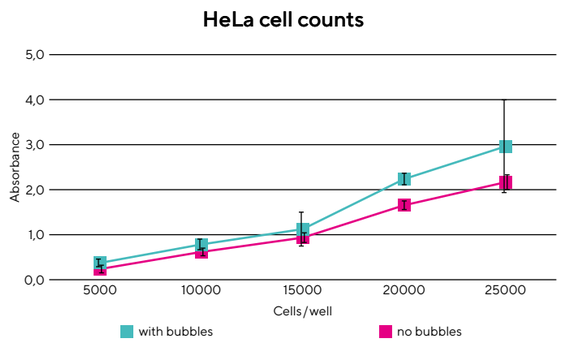

Figure 4: The bubbles affect even distribution of cells and unreliable readouts of absorbance. HeLa cells were pipetted to 96-well plates using either forward (basic) pipetting technique with blow-out in the end or by using reverse pipetting. The results show that there is a significant difference between results obtained by forward pipetting (with bubbles) and reverse pipetting (no bubbles). This proposes that bubbles interfere with even distribution of cells and absorbance based measurement and generate larger deviation between replicates.

Conclusion

Accurate analysis of cell-based assays is dependent on how consistent cells are seeded and distributed in each well throughout the plate. Differences in initial seeding density will cause differences in the overall growth curve which can have a dramatic effect on how cells respond to drug treatment (Niepel et al. 2017). Pipetting errors like poor mixing and bubbles contribute to an uneven distribution of cells and variability from assay to assay. Optimizing pipetting and seeding techniques at the start of the assay is important to produce consistent and accurate results.

To avoid variance caused by pipetting it is important to have a structured routine for cell seeding with gentle mixing by pipetting up-and-down without blowout between each dispensing. It is important work with rapid pace and don't allow the cells to settle before cell seeding. Bubbles can be avoided by using reverse pipetting technique. Working with electronic pipette in multi-dispensing mode speeds up the work and reduces bubbles.

References

1. Veraitch FS, Scott R, Wong JW, Lye GJ, Mason C. The impact of manual processing on the expansion and directed differentiation of embryonic stem cells. Biotechnol Bioeng. 2008; 99(5):1216-29.
2. Niepel M, Hafner M, Chung M, Sorger PK. Measuring Cancer Drug Sensitivity and Resistance in Cultured Cells. Curr Protoc Chem Biol. 2017; 9(2):55-74.