

To study the effects of 2 separate compounds on Horizontal Migration using the Cell-IQ®

Objective

– to investigate the effect of MitomycinC, a DNA interchelating agent, and Y27632, a Rho Kinase inhibitor, on 2 different cell types in a wound-healing assay using the Cell-IQ®.

Material and methods

Cells

HT1080 cells (available from ATCC), plated out at 5×10^4 cells/well
MDA-MB231 (available from ATCC), a breast cancer cell-line, plated out at 2×10^5 cells/well

Growth Media

Dulbecco's MEM 9 (Gibco cat #)
10% foetal calf serum
10 Glutamine (Gibco 100x stock)

Method

Cells were first incubated for 48 hours in Nunclon 48 well tissue culture plates at 37°C to enable attachment and growth to confluence. A 200 µl pipette tip was used to scrape an area of cells away from the confluent monolayer to mimic a wound. The media was changed to growth media + DMSO. The plates were then loaded onto the Cell-IQ® and independent areas for measurement were found in each well by scanning the plate manually. 3 Regions of Interest (ROI) per well per wound edge were chosen for imaging over a 21 hour period (1,2).

Results

HT1080

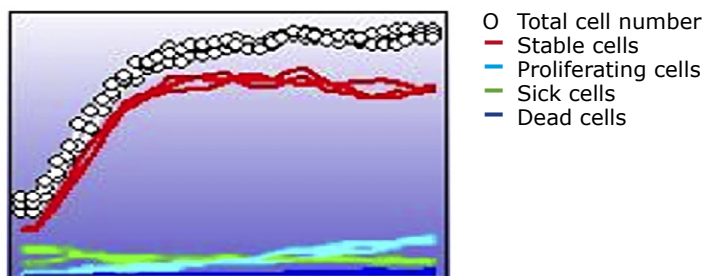


Fig 1. Cell-IQ® representation of triplicate data of HT1080 cells (control) over a 21 hour test – showing steady increase in cell number over time. 50% of wound is covered by migrating cells after 4 hours, and full wound closure is observed after 10 hours.

APPLICATION NOTE

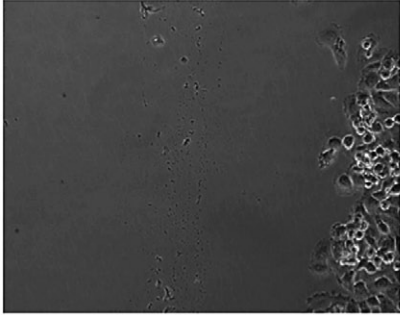


Fig 2. Cells at beginning of test

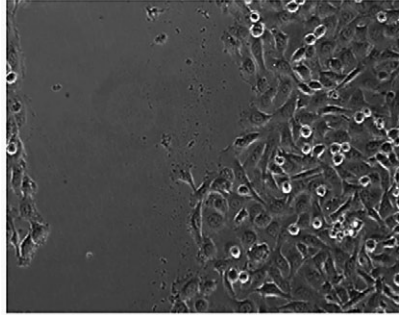


Fig 3. Cells at 4 hours

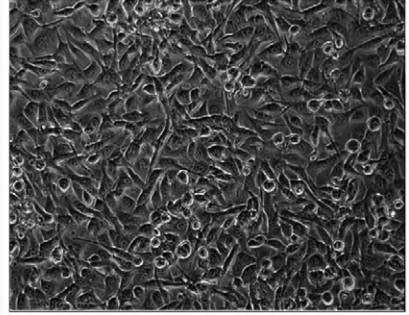
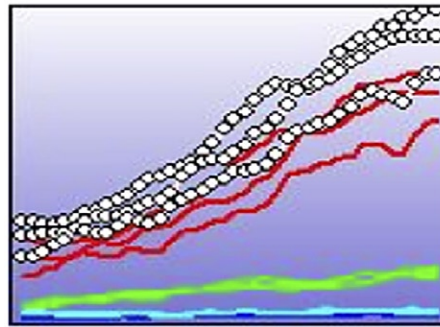


Fig 4. Cells at 10 hours

MDA-MB231



- Total cell number
- Stable cells
- Proliferating cells
- Sick cells
- Dead cells

Fig 5. Cell-IQ® representation of triplicate data of MDA-MB231 cells (control) over a 21 hour test – showing steady increase in cell number over time. 50% of wound is covered by migrating cells after 4 hours, and full wound closure is observed after 10 hours

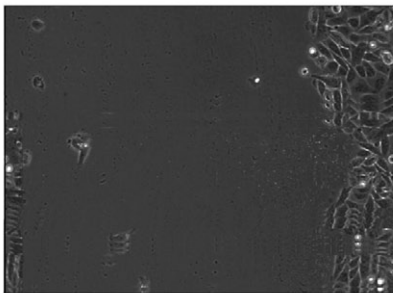


Fig 6. Cells at beginning of test

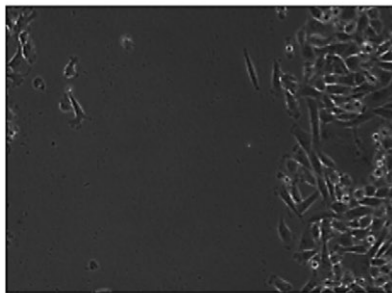


Fig 7. Cells at 4 hours

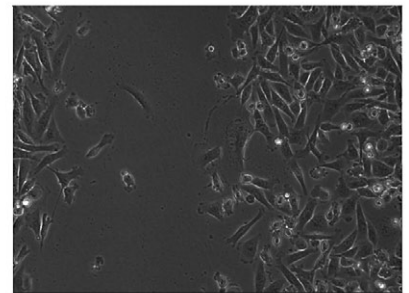


Fig 8. Cells at 10 hours

These are images of the culture during incubation and real-time image capture in the Cell-IQ® system. The Analyser software was trained to recognise the different cell types and measure the number of cells in the ROI at each time point. The figures below represent those numbers, taken from each of the 3 ROIs within each well, to show the consistency of the analysis.

A P P L I C A T I O N N O T E

Summary

- Cell-IQ® provides a very fast and efficient way to measure the rate of migration of cells into a wound space. The simple output gives information on the number of cells moving into the denuded area of the well, as well as the number of cells proliferating and dying during the culture.
- Many replicates can be measured simultaneously – here we have used triplicate measurements – to give greater representation of the culture as a whole, whilst also giving information on reproducibility and reliability of your assay.
- The Cell-IQ® could also be used to generate comparative data on the change in rate of migration in the presence of potential drug candidates, whilst simultaneously monitoring cell proliferation and death.
- Kinetic profile is important for compound analysis and assay development (optimal time-points for analysis for HTS)

References

- (1) Data by kind permission of N. Carragher, AstraZeneca R&D Charnwood, Loughborough, UK.
- (2) L.Scott et al, Molecular and Cellular Biology, Feb 2004, p1540-1559, Vol 24, No4.