dragonfly® discovery
application note

cells dispensed by dragonfly® discovery show normal proliferation, health and apoptotic responses in a range of cell types

introduction

All plate-based assay formats including biochemical, cellular and bead-based require optimisation to identify suitable conditions for robust performance in routine screening applications. We have developed a novel positive displacement dispensing instrument for assay optimisation, which is compatible with 96, 384 and 1,536 well assay plate formats. The wide range of assay volumes is achieved by use of precise, disposable, non-contact syringes for liquid dispensing. Initial work has already demonstrated applicability of this technology for optimisation of biochemical assays.

A common problem for automated dispensers is the risk of compromising the viability and long term health of cells mainly due to shearing and stress effects.

In this study we investigate the compatibility of the system for cell-based assays by direct comparison with hand dispensing. We selected three commonly used cell types in screening, epithelial (A431), hepatic (HepG2) and neuronal (SH-SY5Y). These cell lines dispensed by the dragonfly discovery automated dispenser exhibit normal cell viability, proliferation and apoptotic responses upon activation with staurosporine.

These data sets demonstrate the successful use of dragonfly discovery in cell-based assays.

materials and methods

cell preparation and seeding

Immortalised cell lines of different lineage and host were used, namely A431 (Sigma # 85090402-1VL; epithelial), HepG2 (Sigma # 85011430-1VL; hepatic) and SH-SY5Y (Sigma # 94030304-1VL; neuronal). Each were grown in respective culture media and trypsinised, then resuspended at 10,000 cells/mL in appropriate complete culture medium. Cells were then dispensed (50 µL per well) either using a hand-held repeating pipette or dragonfly discovery automated pipetting system into into 384 well tissue culture plates (Greiner #781091). The outer two wells contained culture medium only to act as an evaporation barrier to prevent plate edge effects interfering with assay performance. Plates were incubated at 37°C, 5% CO_2 until assay intervention was required as described in the following experimental details.

proliferation and cell health

At indicated timepoints, 5 µL of a mixture of Hoechst 33342 (Invitrogen cat# H3570) and propidium iodide (Invitrogen cat# P3566) diluted in phosphate buffered saline (ThermoFisher cat# 14190-094) were added to give working concentrations of 10 µM of each nuclear stain. The plate was then incubated at 37°C, 5% CO_2 for 1 hour and then scanned on mirrorball®. Total cell number was determined for each well by dividing the total area of Hoechst staining by the area of a single Hoechst-stained nucleus. The number of dead cells for each well was determined by dividing the total area of propidium iodide staining by the area of a single propodium iodide-stained nucleus. Propidium iodide stains dead and dying cells as it cannot pass through the intact cell membrane of a healthy cell. A percentage cell viability per well is calculated based on the number of total cells and number of dead cells. Each data point is representative of six replicates within the experiment. Data sets shown are representative of three complete experimental repeats.

Fig 1. dragonfly discovery automated pipettor

caspase 3/7 activity

After overnight incubation at 37°C, 5% CO_2, 5 µL per well of staurosporine (Sigma # S4400) at was added to assay wells to give the required working concentration of drug. The plate was then incubated for 24 hours at 37°C, 5% CO_2. Wells were treated with 5 µL per well of a detection mixture containing CellEvent Caspase-3/7 (Life Technologies # C10423) and Hoechst 33342 (Invitrogen cat# H3570) to give working concentrations of 1µM and 10 µM respectively. The plate was then incubated at 37°C, 5% CO_2 for 30 minutes and then scanned on mirrorball. Total cell number per well was determined from the Hoechst scan and total caspase-3/7 intensity was determined from the green channel. Data was normalised to give a measure of caspase-3/7 activity per cell in each assay well. Each data point is representative of six replicates within the experiment. Data sets shown are representative of three complete experimental repeats.

key benefits

cells dispensed by the dragonfly discovery automated liquid dispenser exhibit comparable responses to cells dispensed by hand when looking at:

- **proliferation profiles**
- **cell viability**
- **apoptosis**

we have demonstrated, the utility of dragonfly discovery to dispense commercially available immortalised cell lines whilst maintaining cell viability and function.

sptlabtech

results

dragonfly discovery dispense technology

In each of the channels (up to 10) there is a tight fitting piston that travels within a pipette barrel, when coupled to the instruments piston rod the positive displacement syringe is formed. The distance and rates of acceleration and deceleration of the piston control how and when liquid is ejected from the tip.

Each channel is fully independent of the others, yet they can all be operated simultaneously, giving rapid, but highly flexible dispensing. This enables complex combination gradients to be set up in high density (up to 1,536-well) microplates, as well as high speed bulk filling of common reagents.

cells dispensed by dragonfly discovery show normal proliferation profiles

The three cell lines A431, HepG2 and SH-SY5Y were dispensed into 384 well cell culture plates either by hand pipette or by dragonfly discovery. Similar rates of cellular proliferation were observed for the two dispensing methods (Fig 2).

cells dispensed by dragonfly discovery show normal viability rates

Following on from monitoring cellular proliferation, the next step was to investigate whether automated dispensing had any adverse effects on cell viability. To this end, the three cell lines A431, HepG2 and SH-SY5Y were dispensed into 384 well cell culture plates either by hand pipette or by dragonfly discovery and the relative levels of cell health were monitored. Cells dispensed by dragonfly discovery showed similar viability to hand-dispensed cells (Fig 3).

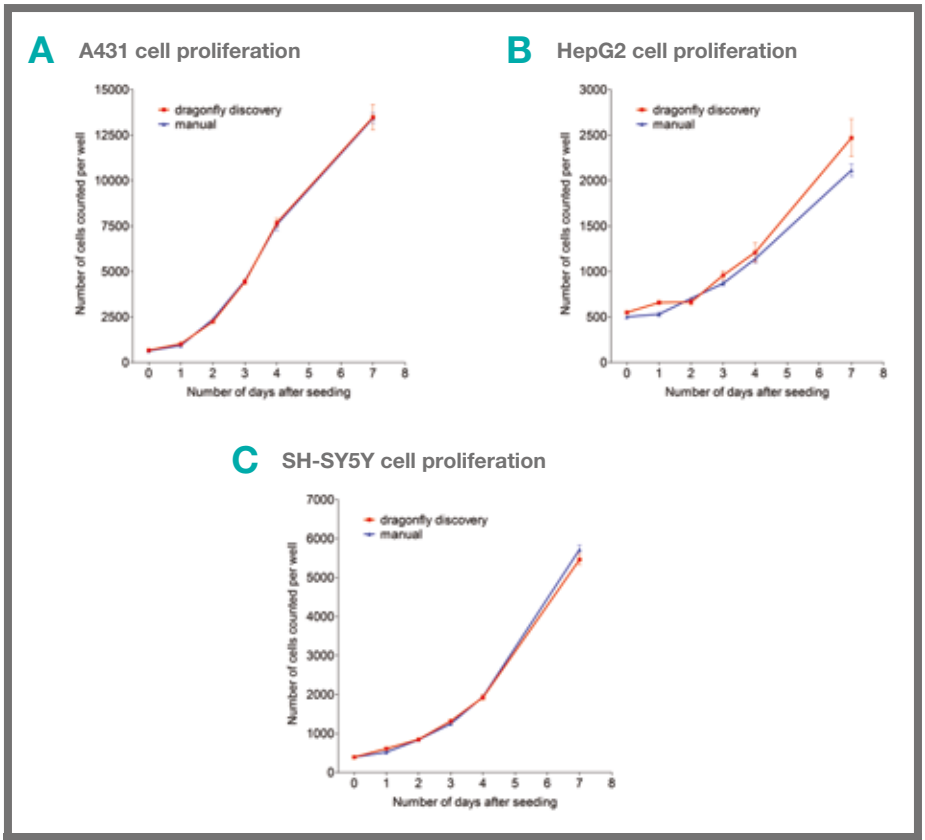

Fig 2. Cells dispensed by dragonfly discovery show similar proliferation to hand-dispensed cells. 50 µl of A431 (A), HepG2 (B) or SH-SY5Y (C) cells in complete culture medium were dispensed per well either by hand or by dragonfly discovery into 384 well cell culture plates. Cell proliferation was measured at indicated timepoints by the addition of Hoechst 33342 to give 10 µM working concentration. Cell number was determined by scanning on mirrorball. Each data point shows mean +/- s.e.m. of six replicate wells and data sets shown are representative of three separate experiments.

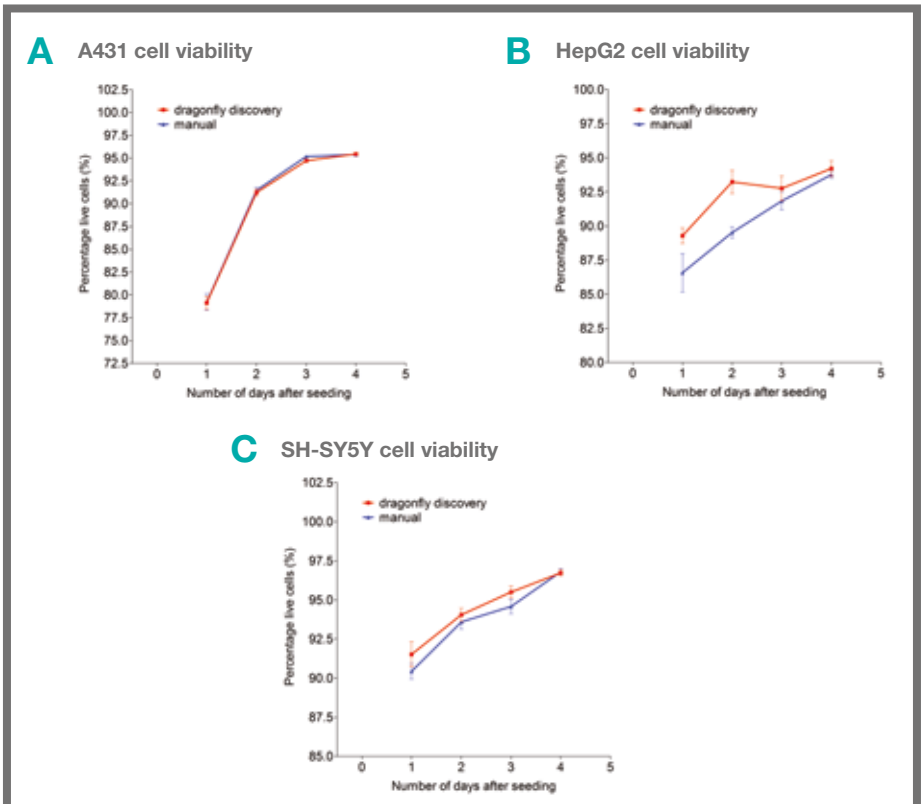

Fig 3. Cells dispensed by dragonfly discovery show similar viability to hand-dispensed cells. 50 µl of of A431 (A), HepG2 (B) or SH-SY5Y (C) cells in complete culture medium were dispensed per well either by hand or by dragonfly discovery into 384 well cell culture plates. Cell viability was determined by the addition of Hoechst and Propidium iodide (giving 10 µM working concentration of each) to the wells at the indicated timepoint. Live/dead cell analysis was determined by scanning on mirrorball. Each data point shows mean +/- s.e.m. of six replicate wells and data sets shown are representative of three separate experiments.

cells dispensed by dragonfly discovery exhibit normal drug-induced apoptotic response

Having shown that basic cell proliferation and viability was similar, the next step was to investigate whether cellular responses behave as expected for cells dispensed by dragonfly discovery.

The act of dispensing cells by dragonfly discovery itself causes no significant activation of the caspase-3/7 apoptotic marker in A431 cells (Fig 4A). The same was also observed with HepG2 and SH-SY5Y cells (data not shown). Furthermore, these cells demonstrate a normal concentration-dependent activation of the caspase-3/7 marker upon treatment with staurosporine (Fig 4B-D). The lack of caspase-3/7 response by HepG2 cells to staurosporine treatment is in agreement with observations in the literature [1] and the same lack of response is observed with HepG2 cells dispsensed by both dragonfly discovery and by hand.

conclusions

Optimisation of experimental conditions is an essential step when setting up new assays to ensure a robust protocol can be identified. In cell-based experiments, it is essential that the methods used to dispense the cells do not themselves influence cellular health or response to external stimuli such as potential drug candidates, as this would compromise the assay reliability. To this end we have compared the cellular health and drug responses of three commonly used cell lines when either dispensed by hand or dragonfly discovery.

Similar rates of cellular proliferation were observed for the two dispensing methods (Fig 2), suggesting no adverse effects on basic cell function due to automated dispense. Indeed when cellular viability was tracked over several days, again there was no significant difference between hand-dispensed and dragonfly discovery dispensed cells (Fig 3).

Having now demonstrated there were no detrimental effects on cell proliferation rates and viability, the next consideration was cellular function. Apoptosis is a commonly studied process which is known to be involved in the pathogenesis of many disease conditions, including cancer, viral infections, autoimmune diseases, neurodegenerative disorders, and AIDS (acquired immunodeficiency syndrome) [2]. One marker for apoptosis is the activation of caspases 3 and 7. One concern for automated cell dispensing via a 0.4mm diameter syringe was that apoptosis may be induced due to exposure to shear forces. However, these data sets clearly show that the carefully controlled dragonfly-dispensed cells show no difference in caspase-3/7 activity relative to hand-dispensed cells (Fig 4A). Moreover, similar caspase-3/7 activation levels were observed for both methods of cell dispense when treated with a concentration range of staurosporine (Fig 4B-D).

Overall, in this study we have shown that cells dispensed by the dragonfly discovery automated liquid dispenser exhibit comparable responses to cells dispensed by hand with regards to:

- proliferation profiles
- cell viability
- apoptosis

We have demonstrated, the utility of dragonfly discovery to dispense commercially available immortalised cell lines whilst maintaining cell viability and function.

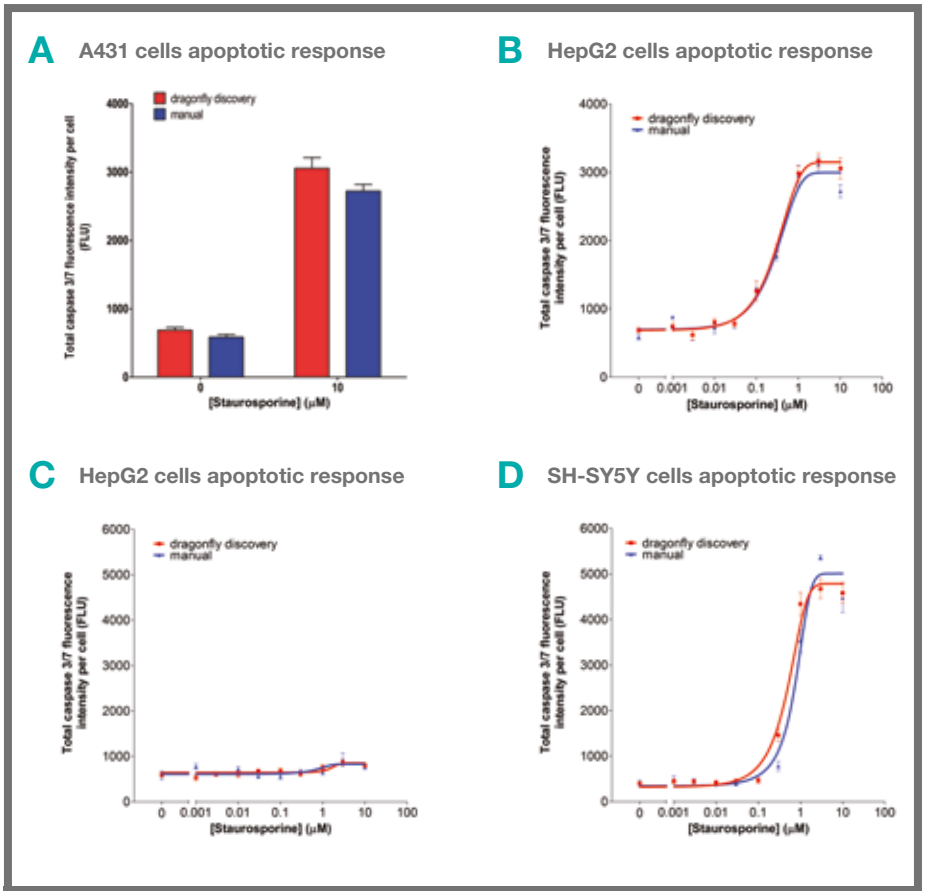

Fig 4. Cells dispensed by dragonfly discovery show similar apoptotic responses to hand-dispensed cells. 50 µL of A431 (A & B), HepG2 (C) or SH-SY5Y (D) cells in complete culture medium were dispensed per well either by hand or by dragonfly discovery into 384 well cell culture plates. No apoptotic response was seen due to dispense in A431 cells (A). Cells were treated with staurosporine for 24 hours (B-D) before staining with Hoechst and CellEvent caspase-3/7 reagent (giving 10 µM and 1 µM working concentrations respectively of each. Caspase-3/7 activity was determined by scanning on mirrorball. Each data point shows mean +/- s.e.m. of six replicate wells and data sets shown are representative of three separate experiments.

references

1. Staurosporine-induced apoptosis in Chang liver cells is associated with down-regulation of Bcl-2 and Bcl-XL. Michela Giuliano Giuseppe Bellavia Marianna Lauricella Antonella D'Anneo Barbara Vassallo Renza Vento Giovanni Tesoriere. International Journal of Molecular Medicine April 2004, Volume 13 Issue 4, Pages:565-571. https://doi.org/10.3892/ijmm.13.4.565

2. Apoptosis in the pathogenesis and treatment of disease. Thompson CB. Science. 1995 Mar 10;267(5203):1456-62.

designed for discovery

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