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

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introduction

All microplate-based biochemical, cellular and bead-based assays require optimisation to identify the ideal combination of conditions for robust screening performance. We have developed a novel positive displacement dispensing instrument, for assay optimisation, which is compatible with 96, 384 and 1,536 well microplate formats. This wide ranging plate compatibility is achieved by use of precise, disposable, non-contact syringes.

Previous work has demonstrated the applicability of this technology for biochemical assay optimisation. In this study we investigate the compatibility of the system for cell-based assays.

A problem commonly associated with automated cell dispensing is cell exposure to stressful shear forces when passing through the dispensing nozzle. When not managed correctly, cells can suffer from compromised viability and long term health implications, which in turn may affect the accuracy of assay reporting.

To investigate the performance of dragonfly® discovery, we selected three cell types that are commonly used in screening; epithelial (A431), hepatic (HepG2) and neuronal (SH-SY5Y) cells.

When dispensed by the dragonfly discovery automated pipettor, these cell types exhibit comparable; cell viability, proliferation and apoptotic responses following staurosporine treatment, to those pipetted by hand.

These data demonstrate successful use of dragonfly discovery for cell-based assays.

1. dispense technology

Fig 1. dragonfly discovery automated pipettor.

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, in addition to high speed bulk filling of common reagents.

2. 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 microplates either by hand pipette or dragonfly discovery. Similar rates of cellular proliferation were observed for the two dispensing methods (Figure 2).

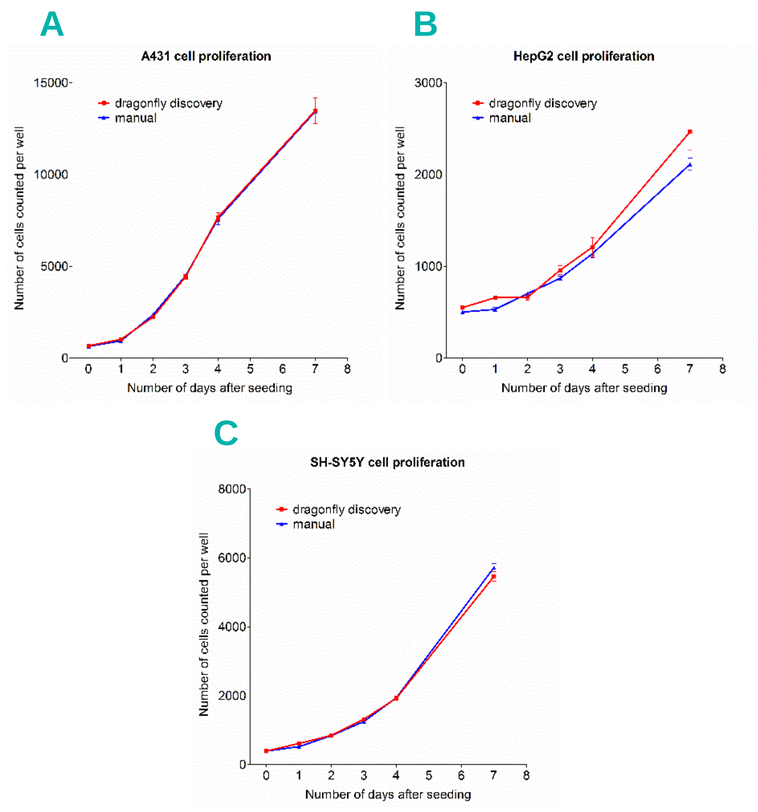

Fig 2. Cells dispensed by dragonfly discovery show similar proliferation to hand dispensed cells. 50 µl of A431 (A), HepG2 (B) or SH-SY5Y (C) cells in complete culture medium were dispensed per well either by hand or by dragonfly discovery into 384 well cell culture microplates. Cell proliferation was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

3. cells dispensed by dragonfly discovery show normal viability rates

The three cell lines A431, HepG2 and SH-SY5Y were dispensed into 384 well cell culture microplates either by hand pipette or by dragonfly discovery. Cells dispensed by dragonfly discovery showed similar viability to hand-dispensed cells (Figure 3).

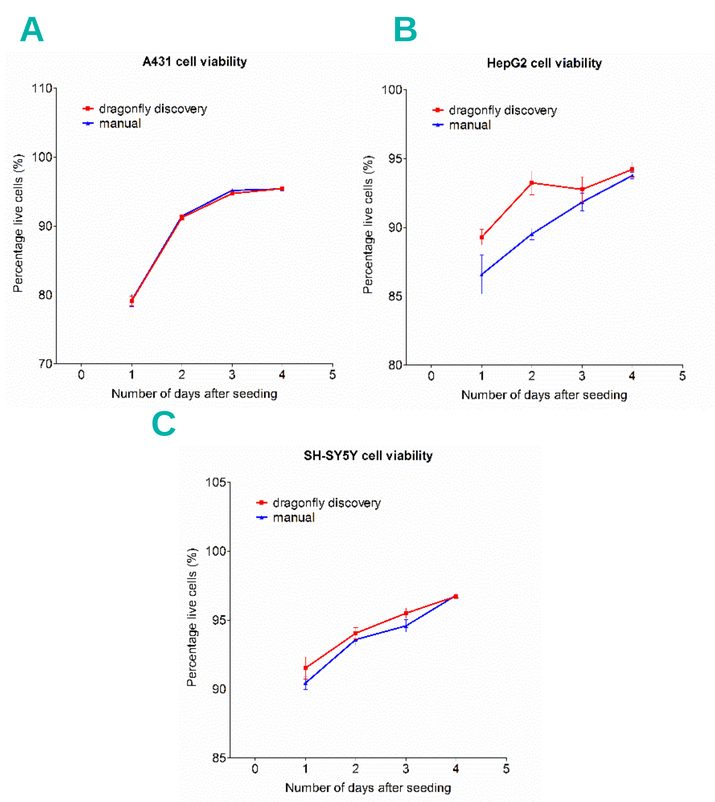

Fig 3. Cells dispensed by dragonfly discovery show similar viability to hand dispensed cells. 50 µl of of A431 (A), HepG2 (B) or SH-SY5Y (C) cells in complete culture medium were dispensed per well either by hand or by dragonfly discovery into 384 well cell culture plates. Cell viability was determined 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 a mirrorball fluorescence cytometer. Each datapoint shows mean +/- s.e.m. of six replicate wells and datasets shown are representative of three separate experiments.

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

Having demonstrated equivalent cell proliferation and viability profiles, the next step was to investigate if cellular responses are triggered by, or behave as expected for cells dispensed by dragonfly discovery.

The act of dispensing cells by dragonfly discovery itself caused no significant activation of the caspase 3/7 apoptotic marker in A431 cells (Figure 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 (Figure 4B-D). The lack of caspase 3/7 response by HepG2 cells to staurosporine treatment is in agreement with observations in the literature (1).

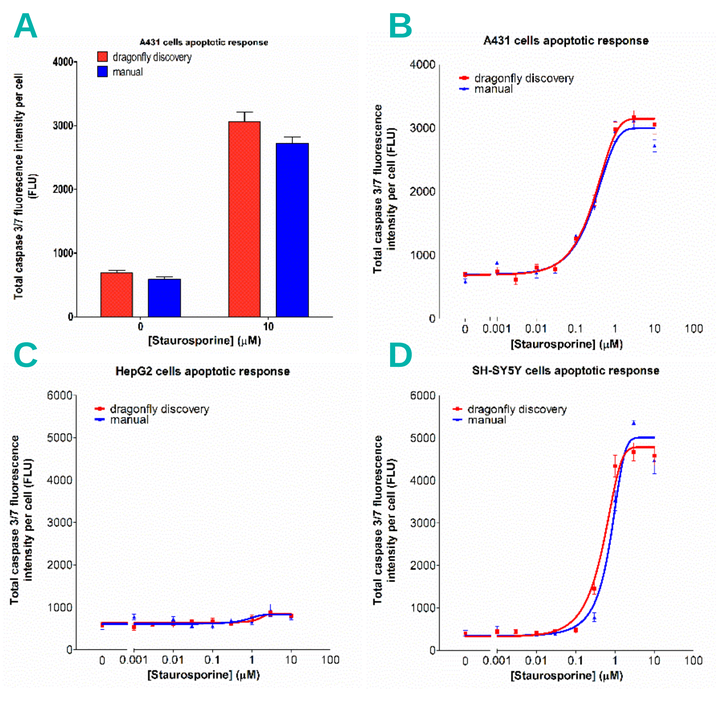

Fig 4. Cells dispensed by dragonfly discovery show similar apoptotic responses to hand dispensed cells. 50 µl of of A431 (A & B), HepG2 (C) or SH-SY5Y (D) cells in complete culture medium were dispensed per well either by hand or by dragonfly discovery into 384 well cell culture plates. No apoptotic response was observed following automated dispensing of 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 fluorescence cytometer. Each datapoint shows mean +/- s.e.m. of six replicate wells and datasets shown are representative of three separate experiments.

5. 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

conclusions

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.