robust and gentle cell plating in 1,536-well microplates using an automated liquid dispenser

dragonfly® discovery

introduction

Cell-based assays play an increasingly important role in drug discovery and HTS due to their increased physiological relevance and are widely considered to serve as better predictors for in vivo studies.

Despite the industry shift towards use of more disease relevant cell types in screens, there are valid concerns surrounding the use of peristaltic dispensers for cell handling that limit or prohibit their use.

To establish if these concerns were alleviated by dragonfly® discovery, an automated, non-contact, positive displacement dispenser, SPT Labtech sought a collaboration with an UK Based Contract Research Organisation (CRO) who provide drug discovery services to a multitude of partners across a wide range of therapeutic areas.

This CRO's HTS infrastructure is made up of fully automated laboratory workcells and standalone small-scale automation, however, automating the setup of cell-based assays remains a key challenge for their team. Some commonly used dispensers carry large reagent dead volumes of several millilitres, which is unacceptable for specialised cells types.

key benefits

- cells dispensed by dragonfly discovery show excellent cell proliferation and viability for a range of cell types
- cell plating was carried out in 1,536-well plates using only 2 µL of reagents
- robust nuclear cell counts were confirmed across all 1,536-wells of the plate 24h after seeding
- the autofeed reservoir module increases the liquid capacity, and consequently cell plating throughput of the dispenser

Other challenges include compromised cell health and viability caused by peristaltic dispensers, uneven cell distribution and the lack of ability to vary reagent conditions in 1,536-well microplates.

As their site maintains over 40 different validated cell lines in continuous culture at any given time, this CRO requires an automated dispense solution that can universally provide robust and gentle cell plating into 1,536-well microplates.

Through collaboration with SPT Labtech, they evaluated the performance of the dragonfly discovery dispenser and its associated auto-feed reservoir module to address these challenges.

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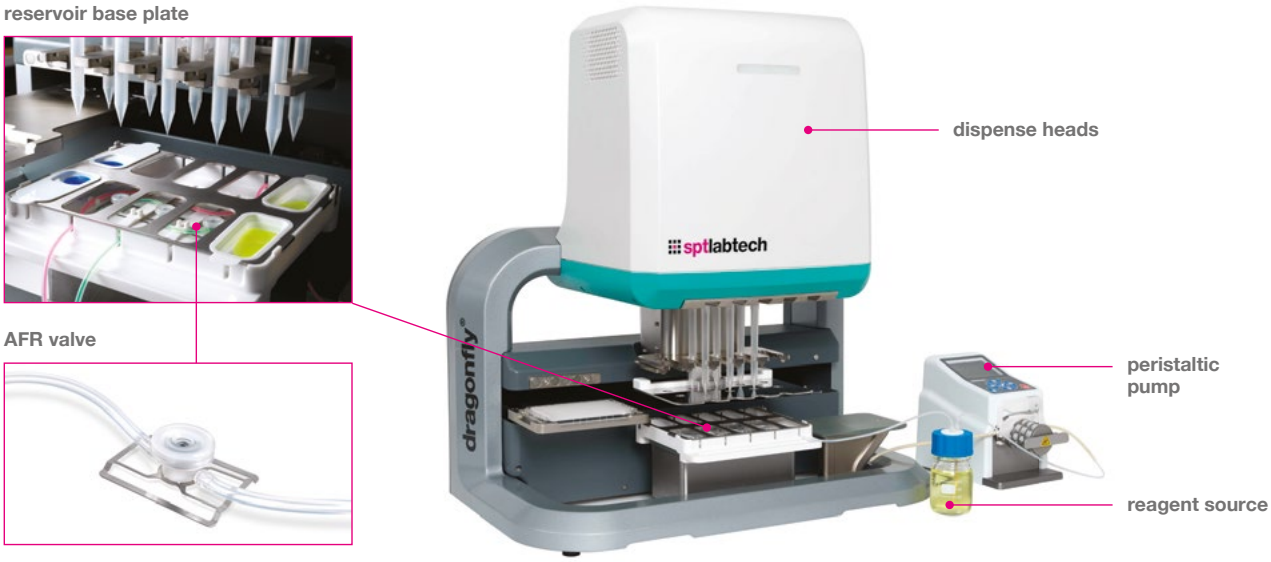

Figure 1: The dragonfly discovery instrument and auto-feed reservoir (AFR) dispense module.

The AFR module is compatible with all dragonfly discovery configurations (3/6/10 heads) and increases the reagent capacity per dispense channel over manual reservoirs. AFRs are based around a single-use valve, from which liquid aspiration occurs. It is made of two silicone seals encased in a polypropylene shell and controls the flow of liquid through the input/output tubing, as well as liquid supply to the tip. A peristaltic pump supplies reagents to the AFR valve from a source container, either in continuous closed loop circulation, or to waste. AFR valves have a spring-loaded metal holder that clips securely onto a reagent tray. It is possible to connect up to three AFR valves in sequence and to mix and match manual and AFR reservoirs.

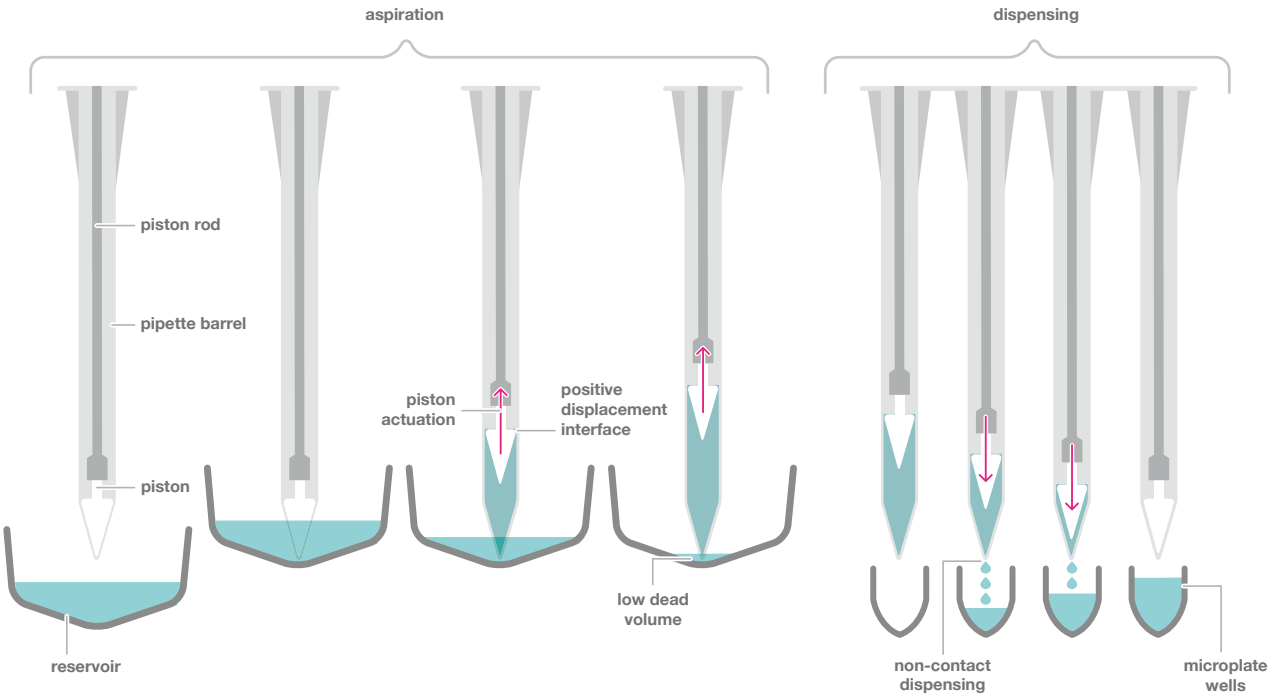

Figure 2: dragonfly® discovery aspiration and dispense mechanism.

Each liquid channel comprises a tightly sealed plunger that travels vertically within a syringe body. When coupled to the instrument's piston rod, the positive displacement syringe is formed. The distance and rates of acceleration and deceleration of each piston rod control how and when liquid is aspirated or dispensed. Each positive displacement syringe is independently controlled.

materials and methods

technology overview

The dragonfly discovery instrument (Fig 1) comprises up to 10 independently controlled dispensing heads inside the top cover. It uses a unique non-contact, positive displacement mechanism to dispense liquid from disposable tips (Fig 2) into 96, 384 or 1,536-well microplates. at speeds of <30 seconds per 384-well microplate or <90 seconds per 1,536-well microplate.

Dispense reagents are supplied from single use liquid reservoirs. For low throughput experiments involving fewer than 5 plates, standard reservoirs (10 mL capacity, 300 µL dead volume) or low dead volume reservoirs (1.5 mL capacity, 30 µL dead volume) are recommended. For high throughput experiments, auto-feed reservoirs (as shown in Fig 1) offer a continual supply of reagents from an offline source without the need for reagent replenishment.

cell culture, plating & analysis

Five cell lines commonly used in different application areas: CHO-Nav (patch clamp screening), CHO-Trk and HT1080 (kinase screening), Neuro2A (neuronal assays) and HepG2 (liver assays) were maintained in live culture according to the cell line vendor's recommendations for growth medium, cell density and environmental control.

On the day of the experiment, the cells were brought into suspension by trypsinization and their concentration was adjusted to 2.5 x 10^5 cells /mL by dilution in the appropriate cell culture medium.

The dragonfly discovery instrument was used to dispense 2 µL (i.e. 500 cells) of each cell suspension into 1,536-well microplates (CellCarrier Ultra, Perkin Elmer 6004550). For each cell line, two replicate plates of cells were seeded: The first plate contained cells drawn from standard dragonfly discovery reservoirs and the second cells drawn from auto-feed reservoirs.

After plating, the cells were grown for 24h at 37ºC, 5% CO_2 in a humidified incubator and then stained for 30 minutes by the addition of 2 µL of Hoechst 33342 (1:5000 dilution, Gibco H3570) and propidium iodide (1:1000 dilution, Thermo Fischer Scientific P3566). The plates were imaged on an InCell 2200 imaging system (GE Healthcare) and analysed in Cell Profiler (www.cellprofiler.org) to determine cell counts, as well as the percentage of viable cells.

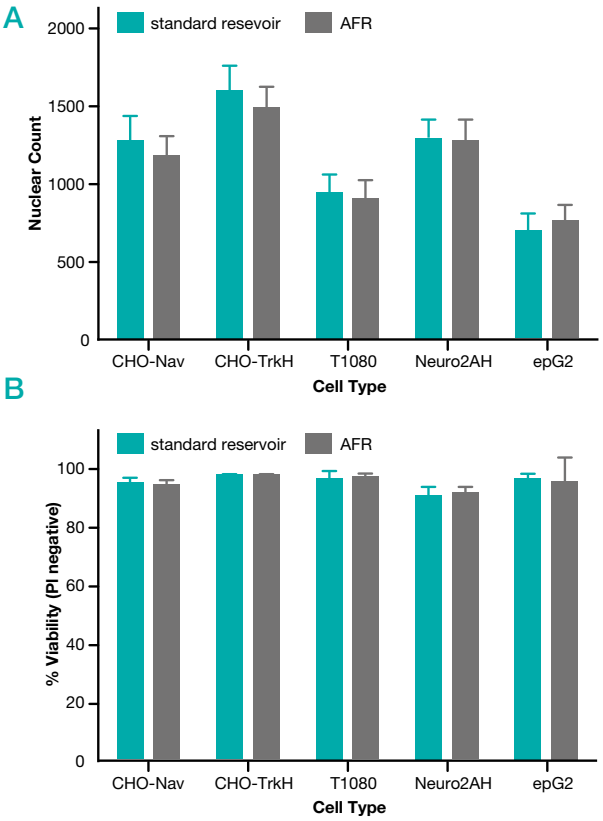

Figure 3. (A) Cell count and (B) % viability 24h after cell seeding with dragonfly® discovery Values represent mean ± SD (n=1536 wells)

results

Figure 3 compares the nuclear count and % viability of cells dispensed with dragonfly discovery for all five cell lines. For each cell line, there was no difference in nuclear count, nor % viability when comparing cells drawn from manual reservoirs to cells drawn from AFR reservoirs. These values were also comparable to those of cells dispensed by peristaltic dispensers.

conclusion

These data sets show that cell plating with dragonfly discovery in either reservoir configuration has no adverse effects on cell proliferation or viability. Additionally, nuclear cell count data after 24h confirm a uniform cell distribution across wells within the plate. Unlike peristaltic dispensing, automated dispensing with dragonfly discovery is compatible with 1,536-well microplates. Overall dragonfly discovery offers a robust, yet gentle, solution to automate cell-based assays.

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