Faster Drug Discovery with Picodroplet Technologies

Automated platforms based on picodroplet microfluidic technologies are one of the most promising tools for improving drug discovery efficiency. By encapsulating single cells in miniaturised, aqueous picolitre compartments, called picodroplets, these technologies provide a high-throughput and sensitive method to identify high-affinity, high-potency drug candidates with better biotherapeutic profiles and faster developability timelines. Automated, picodroplet-based workflows can help to streamline the drug discovery process, increase throughput, and reduce time and operational costs.

Challenges in Drug Discovery

As the demand for new therapeutics surges, the acceleration and optimisation of drug discovery processes have never been more crucial. However, the discovery process remains complex, time-consuming, and inefficient, increasing timelines and development costs.

This efficiency problem is attributed to various causes, one of which is the resource- and labour-intensive nature of screening large cell populations for rare antigen-specific, antibody-secreting cells during drug discovery. Traditional hybridoma-based strategies involve laborious screening efforts that create major bottlenecks in finding lead candidates for progression to antibody optimisation and clinical candidate selection (Figure 1).

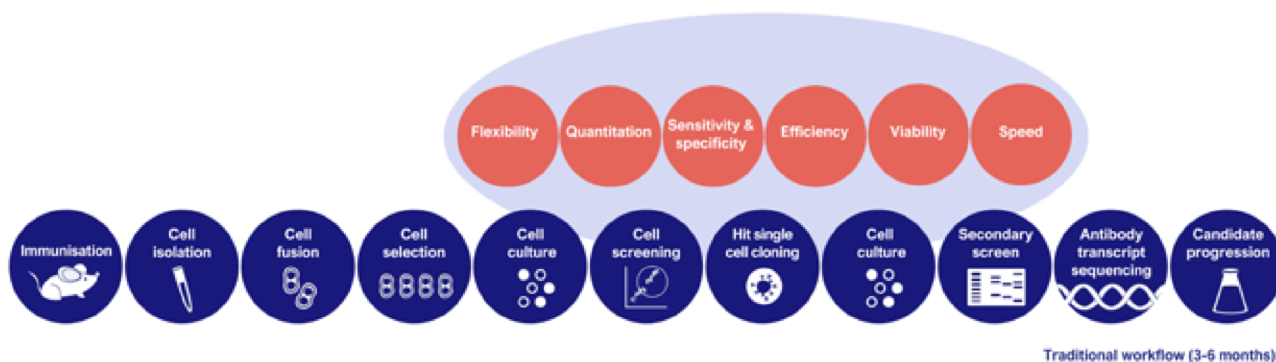

Figure 1. Traditional workflow in hybridoma screening

Advances in automated, high-throughput screening technologies that enable the screening of millions of antibodies to identify new drug candidates can partially overcome the problem. One typical method involves screening the purified B cells directly using flow cytometry, bypassing traditional hybridoma fusion and phage display approaches.

Flow cytometry has the advantage of being very high throughput, and antibodies secreted by B cells can potentially be screened using cold capture, a technique used to prevent the full secretion of antibodies by trapping them at the cell surface. However, this technique produces a representation rather than a direct measurement of the antibody secretion profile by a single cell. There are several other limitations to this screening method, including altered cell function and reduced cell viability. Alternative screening methods include ELISA and Elispot; however, these techniques often need to be executed manually. Consequently, it becomes too costly and time-consuming to analyse large populations[1].

After multiple rounds of screening and selection, the positive cells must then be sub-cloned into monoclonal populations (lead panels) by employing semi-automated methods like cell-in-well imagers and cell sorting; this multi-step approach adds even more complexity and hands on-time, slowing down the discovery process even more[2].

Integrated Drug Discovery Platforms

To remove common bottlenecks and find rare variants faster, biopharmaceutical companies are now looking to picodroplet microfluidics. Picodroplet microfluidic technologies conduct complex multi-step assays with high reliability, cost-efficiency, and throughput in a picolitre-sized aqueous droplet (picodroplet) format. Using this approach, individual cells, or multiple cells in pools, are encapsulated in the picodroplets for high-throughput screening (Figure 2). Picodroplets act as a bioreactor to compartmentalise cells and facilitate growth, eventually trapping secreted molecules such as antibodies, making them easily accessible for characterisation[3].

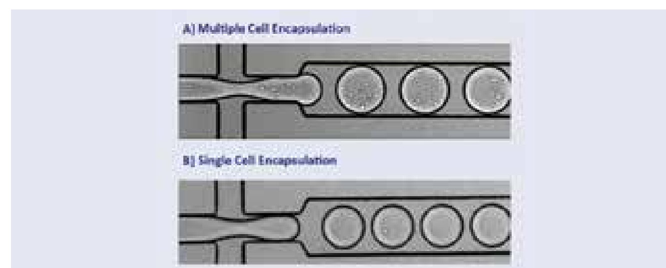

Figure 2. These images show the encapsulation of multiple cells or single cells per picodroplet. A) A large population of cells (>1 million) diluted to a concentration of $1x10^8$ cells/mL in medium resulting in multiple cells per picodroplet. B) Cells diluted to a concentration of $1x10^6$ cells/mL to obtain a population of picodroplets containing single cells.

Emerging fully integrated picodroplet systems offer a unique opportunity to improve the antibody drug process and increase the number of targets that generate biologics. These technologies not only simplify the screening of one to tens of millions of encapsulated cells and their products, but combine the subsequent selective sorting, cell isolation, imaging, and single-cell dispensing stages into an automated platform.

Compared to conventional systems, automated picodroplet systems offer significant advantages in high-throughput single-cell screening, rapid-yet-gentle cell processing, and high-sensitivity quantitative assays. These capabilities facilitate high-throughput research to interrogate larger repertoires and find more functional properties in just days[4]. For example, using a fully integrated platform, researchers can analyse up to 40 million cells (B cells) in two days with each picodroplet containing ~30 cells in the first of a two-run protocol). These systems can also be used to analyse up to 200,000 single cells (B cells or hybridomas) for antigen-specific antibody-secreting cells, isolate high-potency candidates of interest, and directly dispense single cells into individual wells of a microtitre plate, in a single day.

As a result of these advancements, researchers can now 'mine' for the rarest cells that naturally occur in a heterogeneous population to isolate the most valuable antibodies with the greatest antigen-binding affinity and specificity. A process that, when following a traditional, multi-step discovery workflow, can take several weeks (Figure 3).

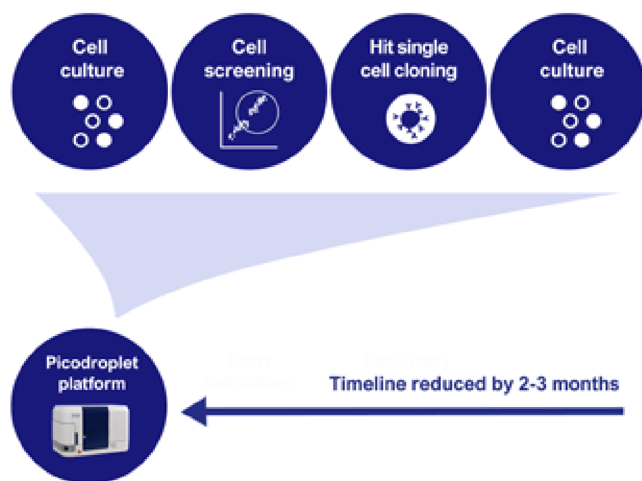

Figure 3. High-throughput screening workflow

Importantly, these platforms maintain the cells in a highly viable state throughout the discovery process, as picodroplets provide a uniquely protective environment to support cell integrity during incubation, shielding cells against shear stress as they flow through the microfluidic channels.

Additionally, the miniaturised format requires much smaller sample volumes, allowing the concentration of the molecules secreted by the cell to accumulate quickly. This provides a more sensitive and accurate measurement of antibody secretion levels to help find rare antibodies with desirable characteristics at a dramatically reduced cost per test.

An Automated, Picodroplet-based Workflow

Fully integrated picodroplet systems consist of five stages; cell isolation, assay, sorting, imaging and dispensing. Streamlined workflows enable researchers to get a complete run-through in a day, starting from the cell sample and ending up with picodroplets dispensed into the wells of a microtitre plate, significantly reducing timelines for the discovery and development of antibody-based therapeutics (Figure 4)[5].

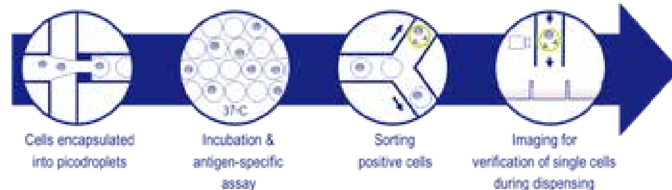

Figure 4. Integration of antigen-specific screening, sorting, imaging and dispensing using a fully automated microfluidic process.

By streamlining the whole discovery workflow into one, easy-to-use instrument, biopharmaceutical companies can remove much of the complexity of the process and critically, switching to a picodroplet-based technology requires very little additional resources, training time, and maintenance.

The stages of an automated, picodroplet-based workflow typically include:

1) Cells encapsulated into picodroplets: The target cell population is prepared in a preferred culture medium and supplemented with an appropriate animal-origin-free antibody-based detection reagent for the selected secretion assay. The cell suspension is then gently processed through microfluidic channels and mixed with an oil containing a biocompatible surfactant, which ensures stable picodroplet formation and encapsulates a single cell (or pools of cells) in each picodroplet.
2) Incubation and secreted protein assay: Approximately two million picodroplets are collected and incubated *in situ* at 37°C to activate cell metabolism and allow the assay signal to develop. Assays may include antigen-specific assays for hybridoma screening or B-cell mining, but this assay format can be adapted and tailored to many different antigen targets.
3) Sorting positive cells: The picodroplets are sorted by fluorescence detection and gating, with positive 'hits' being actively channelled for collection. The population of cells selected for collection can be defined and adjusted according to each specific experiment.
4) Visual verification and dispensing: After completion of the sorting phase, positive picodroplets are selected, imaged, and dispensed to individual wells of a 96- or 384-well microplate prefilled with preferred culture medium.

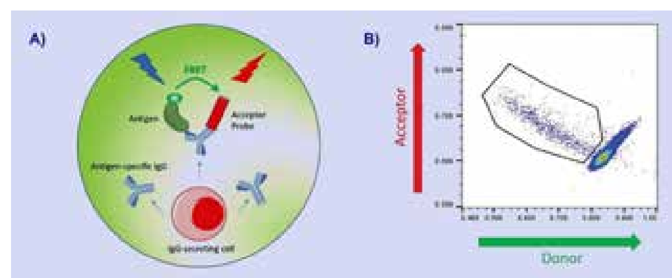

Figure 5. A picodroplet-based antigen-specific assay. (A) Antigen-specific IgG secreted from the encapsulated cell is recognised by the donor conjugated antigen and by the acceptor-conjugated IgG-specific probe. (B) Scatterplot of FRET signal generated from hybridomas screened for secretion of anti-human TNF α IgG.

An example of an antibody discovery experiment which has used the described workflow is included in Figure 5. Josephides *et al.*[3] used an automated, picodroplet-based workflow for the high-throughput screening and selection of antigen-specific clones generated from a mouse immunised with human tumour necrosis factor-α (TNFα). A population of hybridoma cells was analysed with validated detection probes to find TNFα-specific, IgG producing clones (Figure 5A). A subpopulation of cells with a high acceptor-to-donor fluorescence ratio, indicating secretion of human TNFα-specific IgG, was then gated for collection and further analysis, while the remaining picodroplets were diverted to waste (Figure 5B).

The Bottom Line

Researchers can now automate the antibody drug discovery workflow to perform studies with higher sensitivity and speed than conventional systems. High-throughput capabilities enable the screening of hundreds of thousands of individual cells or up to 40 million cells (in pools) to rapidly identify antibody-secreting cells and isolate rare cells secreting antigen-specific antibodies. This enables the discovery of optimal drug candidates from an entire cell library, while ensuring good viability of the cells throughout the process. Overall, picodroplet microfluidic technology presents a compelling opportunity to streamline labour-intensive and inefficient drug discovery, leading to lower operational costs and reduced time to market. In doing so, automated, picodroplet-based technologies address the major challenges faced in the discovery workflow, which are:

- Flexibility: offers adaptable assay design for specific needs
- Measurement: provides quantitative assays of antibody secretion
- Sensitivity and specificity: detects antibodies of interest
- Efficiency: screens the entire cell population at high throughput
- Viability: maintains high levels of cell viability
- Speed: reduces total drug discovery workflow timelines

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Olivia Hughes

Olivia Hughes is a life science writer and digital marketer at Sphere Fluidics. Sphere Fluidics develops and manufactures single cell analysis and monoclonality assurance systems to enable leading-edge research and accelerate biotherapeutic discovery and development. Using propriety picodroplet technology, Sphere Fluidics' flagship product, Cyto-Mine®, integrates isolation, screening, sorting, imaging and dispensing into an automated platform to streamline workflows and capture high-value clones in a single run.

Email: olivia.hughes@spherefluidics.com