Application Note 08

Cell Viability, Proliferation and Outgrowth in Picodroplets

Introduction

Picodroplet technology is a transformative single cell analysis technique based on the rapid encapsulation of individual cells in miniaturized, picoliter-volume, water-in-oil droplets (picodroplets). This approach enables high-throughput screening, gentle cell processing, and high-sensitivity quantitative assays, which allows research to be performed at a single cell level far more efficiently than traditional methods (1).

Each picodroplet serves as a miniature test tube, providing the ability to screen single cells in their own microenvironment with increased precision and sensitivity. This format provides a uniquely protective environment to support cell viability and integrity during incubation, and shield cells against shear stress during subsequent analysis.

Recognizing the potential of picodroplet technology, leading pharma companies are already using this approach to optimize their antibody discovery and cell line development workflows, and to accelerate the discovery of novel biotherapeutics. However, picodroplet technology can be transferable to a wider range of other industries and applications such as genome editing and cell therapy.

By encapsulating a cell within a picodroplet, dilution is minimized, and molecules released by the cell will accumulate and reach a detectable concentration in a relatively short time window. In applications such as antibody discovery, this typically occurs after 1-4 hours of incubation. With regards to genome-editing applications, such miniaturization might help to improve reagent delivery and also help in detection of factors expressed by single cells following a successful editing event.

Picodroplet compartmentalization ensures genetic editing at the single cell level, rather than on a bulk cell population. Also, compartmentalization enables easier detection of low-frequency events, supporting applications such as sorting of rare genome-edited cells. Thus, genome-editing applications in picodroplets can provide multiple advantages. However, it requires prolonged cell incubation in picodroplets to be effective.

This application note demonstrates the feasibility and flexibility of using picodroplet technology for applications that involve prolonged incubation times.

Aims and objectives

This application note shows that:

- Various cell lines can survive in picodroplets for different incubation periods
- Suspension cells proliferate in picodroplets
- Single cells can grow into colonies after recovery from picodroplets

Method

A series of experiments were conducted to evaluate if picodroplets support cell viability, proliferation, and consequential clonal outgrowth of single cells following a 24-72 hour period of encapsulation in picodroplets.

Seven different cell lines, as models for both suspension and adherent cells, were tested in parallel: Raji, THP1, Jurkat and CHO-K1 for suspension lines, and HCT116, MCF7, A431, A549, and HEK293 for adherent lines.

Single Cell Encapsulation

Input cell concentration (2e5 cell/mL) was chosen to optimize the subsequent number of generated picodroplets containing a single cell.

Cells were suspended in culture medium and encapsulated in 450 pL picodroplets using Pico-Surf® 1 surfactant and a Pico-Gen™ 1 Biochip, the emulsion was collected in a 1.5 mL Eppendorf tube then incubated in a CO2 incubator. Cell viability and proliferation were analyzed at four different time points: after cell encapsulation (time 0) and after 24, 48, and 72 hours.

Cell Viability

Cell viability was assessed by staining with Acridine Orange and DAPI (Acridine Orange will stain all living and dead cells, thus providing a total count, while DAPI will stain the dead cells). At each time point, a sample was taken from the emulsion, and the picodroplets

were broken using Pico-Break™ - a reagent used to reduce picodroplet stability. Part of this cell suspension was subsequently labelled with Acridine Orange and DAPI, and analysed using a Nucleocounter NC-3000 (Figure 1). Based on this analysis, an unstained part of the sample was seeded in 96-well plates to be evaluated for outgrowth.

As shown in Figure 1, after 24 hours all but one line (A549) still showed viability over 90%; At the 48 hour time point viability starts to decline for more lines, with the adherent lines A549, A431 and MCF7 being the most affected. After 72 hours of incubation in picodroplets, the trend is still followed, with viability from A549, A431 and MCF7 falling below 40%; Raji, HCT116 and Jurkat show viability around 60%, while THP1, CHO-K1 and HEK 293 show viability of 80% and above. These experiments demonstrated that, following encapsulation in picodroplets and incubation for up to 72 hours, cell viability is affected differently depending on the cell line, with suspension lines showing a better survival in general than adherent ones. Yet, most cell lines exhibited relatively good survival ability in picodroplets for 24-48 hours, demonstrating potential feasibility for applications requiring prolonged incubation. We propose that the observed loss in cell viability is mainly caused by a depletion of nutrients

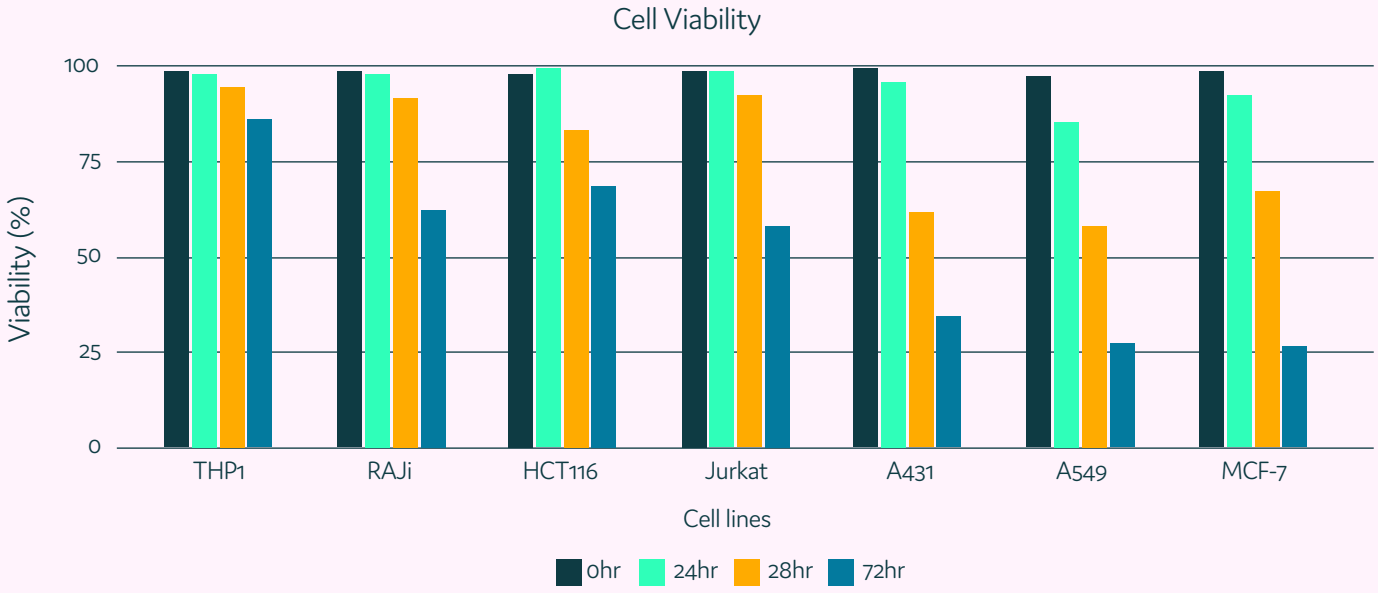

Figure 1. Cell viability in picodroplets.

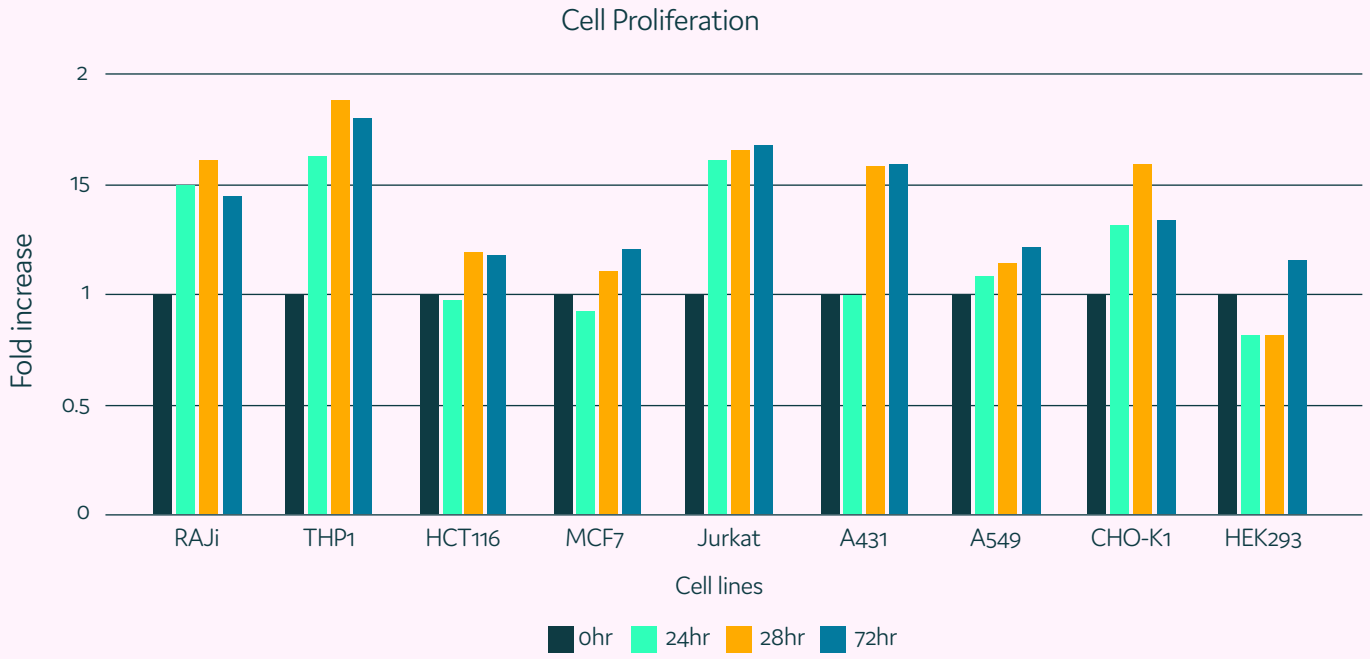

Figure 2. Cell proliferation in picodroplets.

Cell Proliferation

Using the count from the cell viability assay, the concentration of surviving cells was compared to the starting concentration, to determine if proliferation occurred within the picodroplets. The results revealed an increase in cell concentration, especially for the suspension cells Raji, THP1, Jurkat and CHO-K1, demonstrating that picodroplets provide an environment that could support proliferation for certain cell lines (Figure 2). This possibility can provide a basis for further method development that will support generation of a "clonal population" already inside picodroplet.

Cell Outgrowth

After breaking the emulsion and measuring cell viability and cell count, single cell cloning was performed via limiting dilution. Single cells were individually seeded, and subsequently allowed to culture in the incubator for 3 weeks, when the number of grown colonies was counted.

At basal conditions, some lines had a better capability of growing from single cells, as indicated by the starting counts between 70% and 100% (Figure 3). All the cell lines examined still showed good outgrowth, albeit lower than at time 0, after 24 hours in picodroplets, when we found between 50% and 70% of grown

colonies. This changed dramatically for the subsequent time points: While THP1, HCT116 and Jurkat showed sufficient-to-good colony outgrowth after 48 hours (~70%, 45% and 70% respectively) and 72 hours (~55%, ~30% and ~30% respectively), other cell lines behaved differently: A549 and MCF7 showed still a sufficient outgrowth after 48 hours (~30%), but extremely low outgrowth was observed after 72 hours for A549, while ~15% was observed for MCF7. The last two cell lines, Raji and A431, showed very low outgrowth at 48 hours and very little-to-none after 72 hours.

These experiments demonstrated that, following encapsulation in picodroplets and incubation for up to 72 hours, cells still have the capability of growing and creating clonal populations; however, the performance can be extremely different depending on the cell line of interest, with some being able grow at a satisfactory level even after 72 hours, while other showing a dramatic change already after 48 hours. It must be noted, though, that all the experiments were done using standard culturing methods, so development of proprietary cloning media and conditions should enable optimization of the outgrowth for more fragile cells.

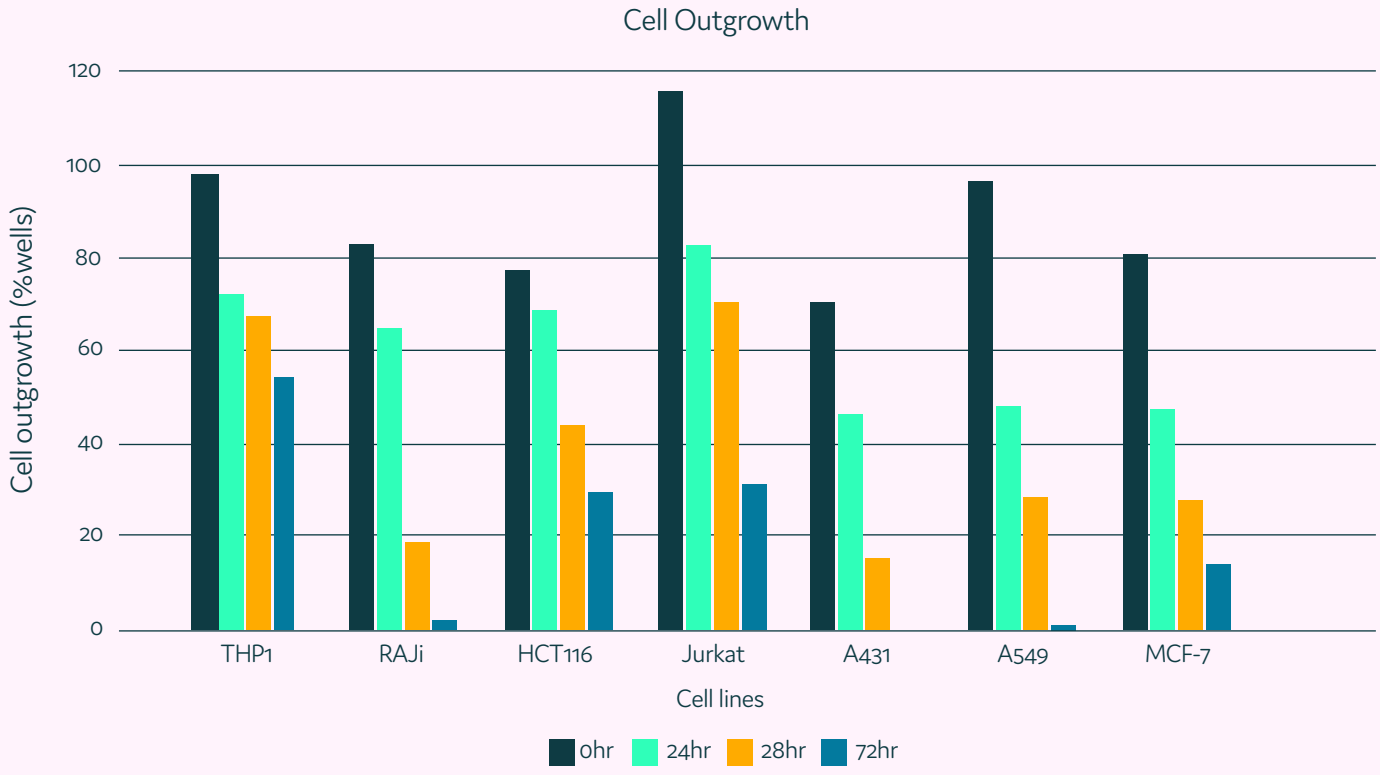

Figure 3. Cell outgrowth after incubation in picodroplets.

Conclusions

Overall, this study reinforces the flexibility of picodroplet technologies, illustrating that picodroplets can support applications requiring a prolonged incubation of encapsulated cells; depending on the cell line, cells can be tested, retrieved and seeded for clone outgrowth after up to 72 hours in picodroplets.

This technique can be used for a wide range of applications including identifying single cells that produce the highest antibody concentrations, isolating rare cells from large populations, and undertaking genome-editing analysis at the single cell level.

References

1. Josephides, D. et al. (2020). Cyto-Mine: An Integrated, Picodroplet System for High-Throughput Single-Cell Analysis, Sorting, Dispensing, and Monoclonality Assurance. SLAS TECHNOLOGY: Translating Life Sciences Innovation. https://doi.org/10.1177/2472630319892571

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