Oribiotech

Unlocking Faster and Better Biology, Cell Therapy Process Development with IRO®: Fluid Dynamics and Mixing Flexibility.

1. Introduction

IRO is a fully flexible manufacturing platform capable of supporting a broad range of cell therapy workflows. Key to this flexibility is its adaptable mixing modes that can be tailored to create distinct bioprocess environments. For example, where modulation of cell-cell interaction or aggregation is required, mixing conditions can be adjusted to support the needs of both the process and the biology. This level of control stands in contrast to many established cell therapy platforms, where protocols are often fixed, adjustments are restricted, or the systems simply lack the capability to generate varied cell culture environments.

Cell therapy process development typically uses one-factor-at-a-time (OFAT) and design of experiment (DoE) approaches to optimize key parameters and understand interactions. These methods are applied within a Quality by Design (QbD) framework to define critical process parameters, establish a robust operating space, and implement a control strategy. However, relying on these methods alone often requires extensive testing, which can be impractical given time and resource constraints such as limited patient material availability.

Ori offers a novel approach where fluid dynamics characterization is used to accelerate process development. By enabling targeted biological testing and helping define the optimal operating space for mixing parameters, this approach narrows the range of conditions requiring biological evaluation. This reduces the number of experiments needed, saving time, cost, and resources while accelerating development timelines.

This case study demonstrates how the IRO platform enables rapid and flexible process development, supporting cell therapy processes with contrasting requirements on a single platform. Rocking mixing parameters were systematically optimized using fluid dynamics–based mixing characterization, including dye mixing, solids suspension, and particle image velocimetry, to narrow the biological testing space and rapidly identify robust, optimal operating ranges.

2. Flexible Mixing Control to Modulate Cell Concentration and Dispersion

2.1. Fluid Dynamics Characterization of Rocking Mixing in IRO®

The IRO platform supports a wide range of cell culture working volumes, from 50 mL up to 1000 mL, and offers multiple mixing modes to suit different phases of a CAR-T manufacturing workflow. These include static culture, rock mixing, and compression mixing. Static and rocking modes are primarily used at lower working volumes during early process steps such as activation and transduction, while compression mixing is better suited to higher volumes during later expansion phases. In this case study, the focus is on rock mixing, which is most relevant at lower working volumes.

Rock mixing generates wave-driven fluid motion that enhances gas–liquid mass transfer at the surface while providing convective mixing throughout the culture. IRO's rocking parameters can be adjusted to create different hydrodynamic environments, enabling control over cell distribution, effective cell density per surface area, and shear exposure. This flexibility also supports uniform suspension of insoluble components, such as activation reagents, as well as nanoscale particles suspended in solution, like viral particles. As a result, these features make IRO well suited for key CAR-T steps, including activation and lentiviral transduction.

Fluid dynamics characterization was used to quantitatively link rocking conditions to mixing behavior, flow regime transitions, particle suspension, and shear exposure. The objective was to develop a mechanistic understanding of how agitation influences cell distribution and interaction, and to use this insight to narrow the experimental space required for biological testing.

Mixing performance was assessed using an automated colorimetric method combined with image processing to measure the mixing time, t_m, across a range of rocking rates. These data were presented as the dimensionless mixing number, $N{\cdot}t_m$, where N is the rocking rate. Analysis of the mixing number revealed distinct flow regimes across the tested operating range (Figure 2.1). At Reynolds number, Re ≈ 1000, the mixing number reaches a constant minimum, indicating the start of the turbulent flow regime. Below this threshold, the system operates within the transitional flow regime, which was subdivided into early (i.e. 100 < Re < 500) and late (i.e. 800 < Re < 1000) transitional flow based on qualitative dye motion analysis.

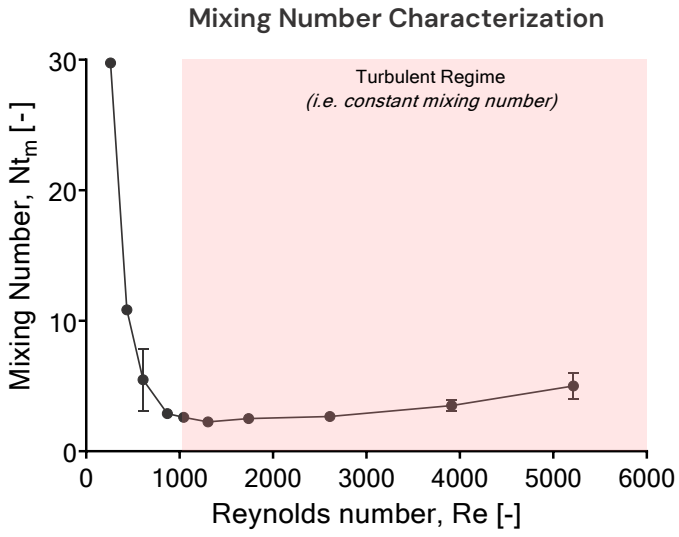

Figure 2.1: Dimensionless mixing number ($N{\cdot}t_m$) as a function of Reynolds number, showing the onset of fully turbulent flow.

To characterize cell and bead suspension behavior, experiments were performed using microparticles specifically selected to model different cellular states during processing.

- Silver-coated hollow glass particles with similar size and density to T cells, which do not aggregate, were used alongside Dynabeads to model non-activated cells and activation reagents.
- Polystyrene particles with similar size and density to single cells, but a tendency to aggregate, were used to represent activated cell populations comprising a mixture of single cells and aggregates.

In the transitional flow regime, rocking mixing promotes local particle concentration, allowing cells and Dynabeads to co-localize within the culture. This behavior is illustrated in Figure 2.2A, where single-cell-like particles and Dynabeads form distinctly colored bands at the same location. In contrast, the turbulent regime disperses all particles, resulting in a uniform suspension with no visible banding (Figure 2.2B).

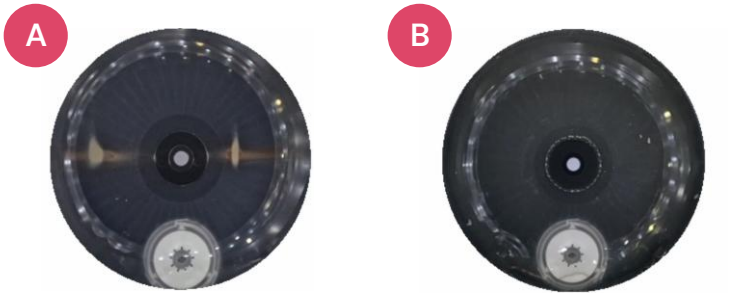

Figure 2.2: Suspension behavior of single-cell-like particles and Dynabeads during rocking mixing: (A) transitional regime (concentrated mode); (B) turbulent regime (dispersed mode).

When aggregates are present, early transitional flow concentrates both single particles and aggregates (Figure 2.3A). As the flow enters the late transitional regime, some single particles remain concentrated (seen as bands) while aggregates begin to disperse (Figure 2.3B). Under fully turbulent conditions, all solids are uniformly dispersed and only aggregates remain visible (Figure 2.3C).

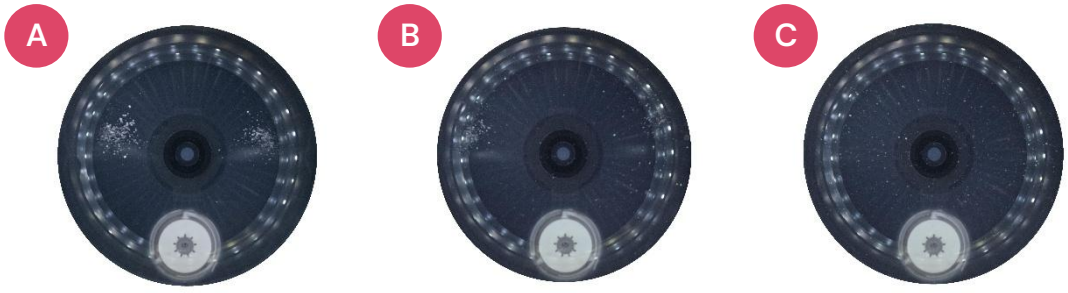

Figure 2.3: Suspension behavior of single-cell-like particles and aggregates: (A) early transitional flow regime; (B) late transitional flow regime; (C) turbulent flow regime.

Beyond mixing and suspension behavior, the mechanical forces experienced by cells are an important consideration. Particle Image Velocimetry (PIV) was used to characterize shear levels within the bioreactor across different rocking conditions. Instantaneous shear rate contour maps at different phases of a rock cycle, overlaid with velocity streamlines (Figure 2.4) show that the highest shear occurs when the free-surface wave reaches the edge of the bioreactor and is reflected in the opposite direction to the main flow. This results in momentary higher shear rates (seen in red), which are localized and decay quickly, while shear levels in the main flow remain low.

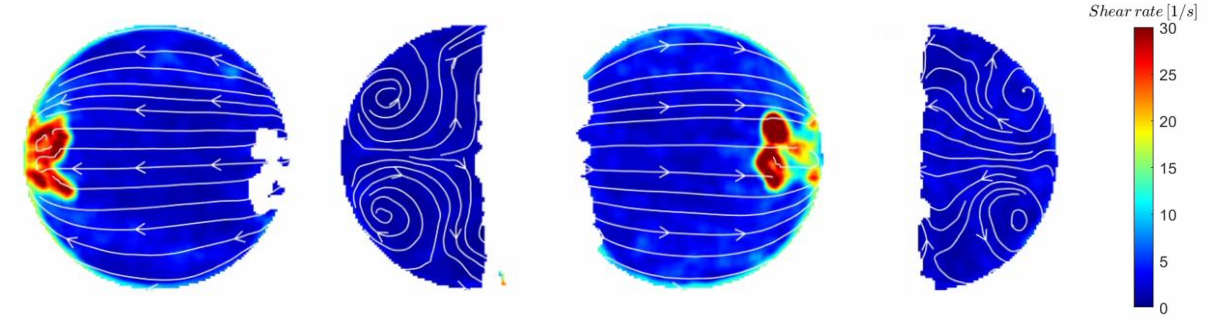

Figure 2.4: Contour plots of the instantaneous shear rate overlaid with velocity streamlines at different phases of a single rocking cycle.

To capture the overall hydrodynamic stress experienced by cells, the mean instantaneous shear rate in the plane was averaged over time to yield a single representative value for each condition (Figure 2.5). As agitation rate increases, the time-averaged shear rate rises, with mechanical forces becoming increasingly adverse to cell growth and viability, particularly in the turbulent regime (shown in Figure 2.5)

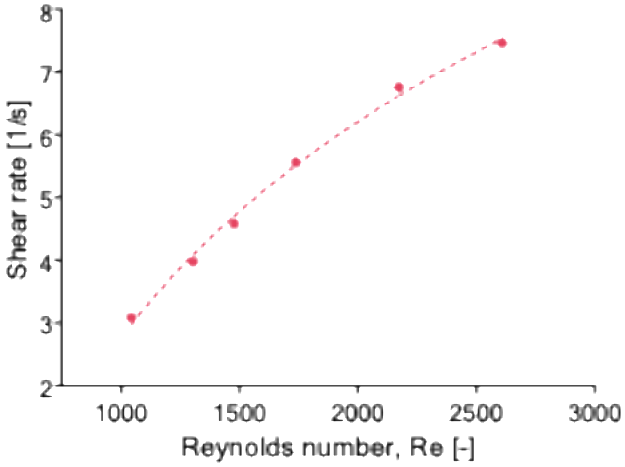

Figure 2.5: Time-averaged shear rate as a function of Reynolds number.

Overall, rock mixing in the IRO® bioreactor enables deliberate control of cell distribution, from localized concentration to full dispersion. When combined with fluid-dynamics-based characterization of mixing and shear, this provides a flexible and predictable framework for tailoring the culture environment to different process phases.

2.2. Modulating Local Bead-Cell Density Through Transitional Flow

Fluid dynamics characterization of the IRO® platform shows that rocking parameters can be adjusted to generate distinct flow regimes that influence how cells and beads distribute at low working volumes. At 50 mL, operating within the transitional regime creates gentle fluid recirculation that promotes local concentration of non-soluble activation reagents (such as CD3/CD28 Dynabeads®) and T cells (as shown in Figure 2.2). This behavior effectively increases the local cell and bead concentration, thereby increasing the likelihood of bead-cell contact.

To assess how these flow-driven concentration effects impact activation performance, healthy donor T cells were seeded and activated with Dynabeads at a 3:1 bead-to-cell ratio, followed by Day 1 lentiviral transduction (anti-CD19 CAR, MOI 0.5). Two activation phase mixing strategies were compared between Day 0-Day 1, alongside an industry standard static control:

- **Concentrated Mode:** agitation parameters selected to operate within the transitional regime, promoting localized bead and cell accumulation.
- **Dispersed-Static Mode:** an initial turbulent phase to establish uniform distribution, followed by static culture to maintain dispersion.

Importantly, both conditions used identical mixing parameters during the transduction and expansion phase, ensuring any observed differences in performance were driven by the activation phase mixing strategies.

Cultures operated in the concentrated mixing mode showed greater T cell expansion than those run in the dispersed/static mode and the industry standard static control (Figure 2.6A). Transduction efficiency was comparable between the two IRO modes (~70%) and both were significantly higher than the industry standard control (~35%). As a result, the concentrated mode produced the highest overall CAR+ cell yield at harvest (Figure 2.6B).

Overall, these findings show that tuning IRO mixing during the activation phase to purposefully increase local bead and cell density provides a practical approach to improving T cell processes with non-soluble activation reagents.

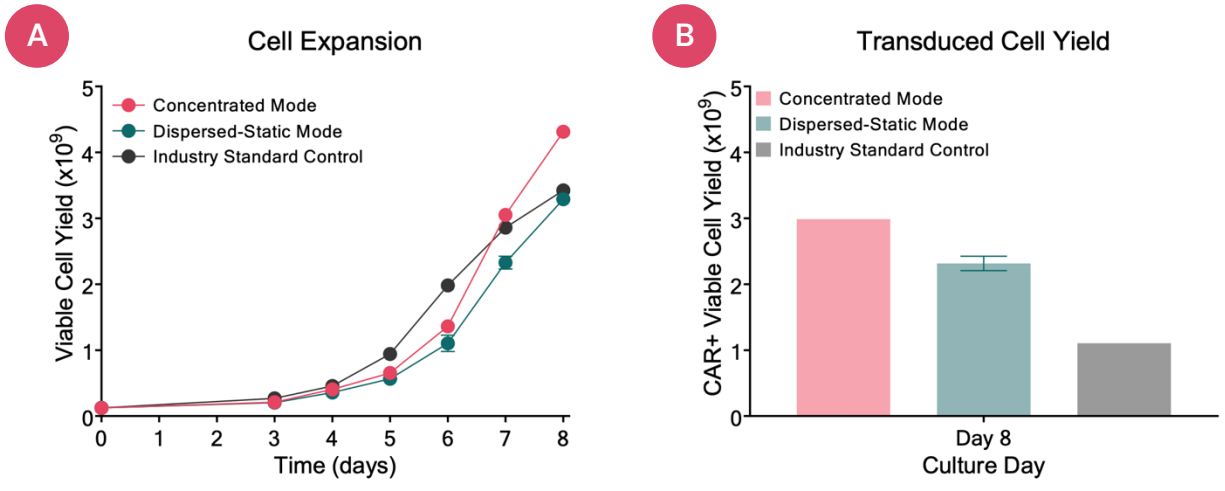

Figure 2.6: Impact of activation-phase mixing regimes on cell and bead distribution and CAR-T production. A) Viable T cell expansion under concentrated versus dispersed IRO operating modes, compared with an industry standard static control. B) CAR+ viable cell yield at Day 8 harvest.

2.3. Leveraging Cell Dispersion to Enhance Lentiviral Transduction

Building on the mixing characterization in Section 2.2, this section examines how IRO®'s ability to shift between cell-concentrating and cell-dispersing states can be applied during lentiviral transduction. The study explores how changes in cell dispersion during transduction affect both transduction performance and subsequent cell expansion.

Healthy donor T cells were activated using TransAct and transduced with a CD19 CAR lentiviral vector on Day 1 at an MOI of 0.5. During the transduction phase (Day 1–Day 2), mixing was adjusted using fluid-dynamics-informed operating states, as outlined below:

- **Concentrated mode:** laminar to early transitional regime, with pronounced cell concentration, long mixing times, and low shear exposure.
- **Partially concentrated mode:** late transitional regime, showing partial dispersion with residual concentration zones and low shear exposure.
- **Fully dispersed, moderate shear mode:** turbulent regime, with uniform cell dispersion, short mixing times, and moderate shear exposure.
- **Fully dispersed, high-shear mode:** turbulent regime, maintaining dispersion but with elevated shear exposure.

Cell expansion varied across the tested operating states (Figure 2.7A). The partially concentrated and fully dispersed, moderate-shear conditions supported the strongest growth, while the concentrated mode showed reduced expansion relative to these conditions. The fully dispersed, high-shear mode resulted in the poorest expansion.

Transduction efficiency increased as conditions shifted from concentrated to fully dispersed states (Figure 2.7B). When combined with expansion, transduced cell yield revealed a clear balance between dispersion and shear (Figure 2.7C). The fully dispersed, moderate-shear condition delivered the highest CAR⁺ cell yield by combining strong expansion with elevated transduction efficiency. In contrast, higher-shear dispersion reduced overall yield due to impaired growth, while more concentrated modes were constrained by lower transduction efficiency.

Overall, these results demonstrate that IRO enables controlled tuning of cell dispersion and shear during transduction to align mixing behaviour with biological requirements. Operating near the onset of turbulence provides a practical balance between enhanced transduction and sustained expansion, highlighting mixing-state control as a powerful lever for accelerating CAR-T process development.

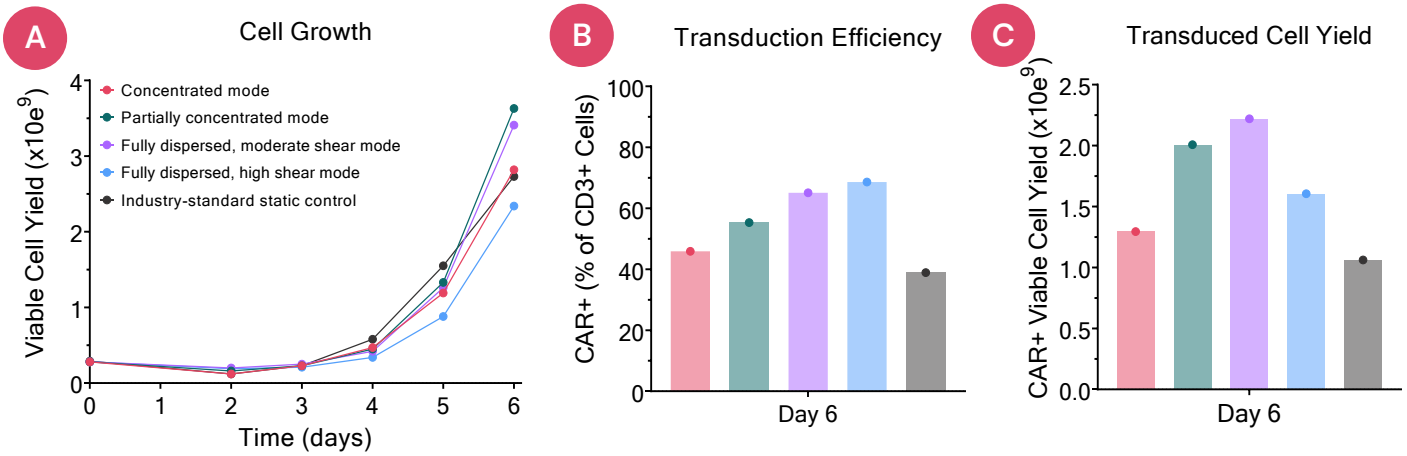

Figure 2.7: Impact of mixing-driven cell suspension state during lentiviral transduction on CAR-T performance in IRO. (A) (A) Viable cell expansion profile under different fluid-dynamics-informed operating states (B) Transduction efficiency following CD19 CAR lentiviral transduction on Day 1 (MOI 0.5). (C) Transduced (CAR⁺) cell yield at Day 6 harvest.

3. Conclusion

Flexible Mixing unlocks Accelerated Process Development with IRO®

This case study shows how the IRO platform's rocking-based mixing can be tuned to suit different biological processes. By adjusting rocking conditions, the system can switch between cell-concentrating and cell-dispersing regimes to support both T cell activation and lentiviral transduction.

Two processes with different activation methods were assessed: Dynabeads (insoluble, high-density beads) and TransAct (soluble, nanoscale reagent). For Dynabeads, mixing was tuned to locally concentrate beads and cells, increasing contact frequency and improving activation. Dynabeads readily settle under low-agitation conditions, making them amenable to concentration-based strategies. In contrast, TransAct and lentivirus are nanoscale particles suspended in solution and remain evenly distributed. Under cell-concentrating mixing conditions, only the cells, not the viral particles, would become locally concentrated. This would diminish effective transduction. Therefore, mixing for the TransAct process was tuned to keep cells dispersed, maintaining consistent contact with soluble reagents and viral particles while minimizing shear stress.

IRO's Fluid dynamics characterization provides a mechanistic understanding of flow regime, particle distribution, and shear exposure, directly linking physical mixing behavior to biological outcomes. These insights enable the rational selection of operating conditions without reliance on extensive biological screening. As summarized in Table 4.1, this case study, this physics-led approach reduced the number of biological runs required to identify the optimal operating window by 60-90% compared with conventional OFAT and DoE strategies.

Overall, the combination of IRO's exceptional mixing flexibility and fluid dynamics-driven process insight delivers faster process development at lower cost for cell therapy manufacturers. Critically, it also enables superior biological performance, achieving more than a two-fold increase in transduced cell yield compared with commonly used platforms, representing best-in-class results for cell therapy processing.

Table 4.1: Comparison of biological run requirements to characterize and select mixing parameters for a given culture volume in a CAR-T process under different process development strategies.

Process Development Approach	Mixing Design Space Explored	Estimated Biological Runs*
One-factor-at-a-time (OFAT)	Sequential exploration of rocking speed, start angle, and stop angle across their full operating ranges (one parameter varied at a time).	~25-50 runs
Design of Experiments (DoE)	Rocking speed × start angle × stop angle across full ranges *(continuous multidimensional design space)*	~325 runs (Full-factorial)
Fluid dynamics–guided (IRO)	**Design space narrowed to a targeted window, with rocking rate and angle selection guided by fluid dynamics characterization.**	**<10 runs**

*Estimated run numbers assume 13 rocking speed levels and 5 start/stop angle levels. OFAT (~25 runs) does not capture parameter interactions; full-factorial DOE would require ~325 runs (13 × 5 × 5). OFAT and DOE values are indicative only; the fluid-dynamics-guided value reflects actual runs performed.