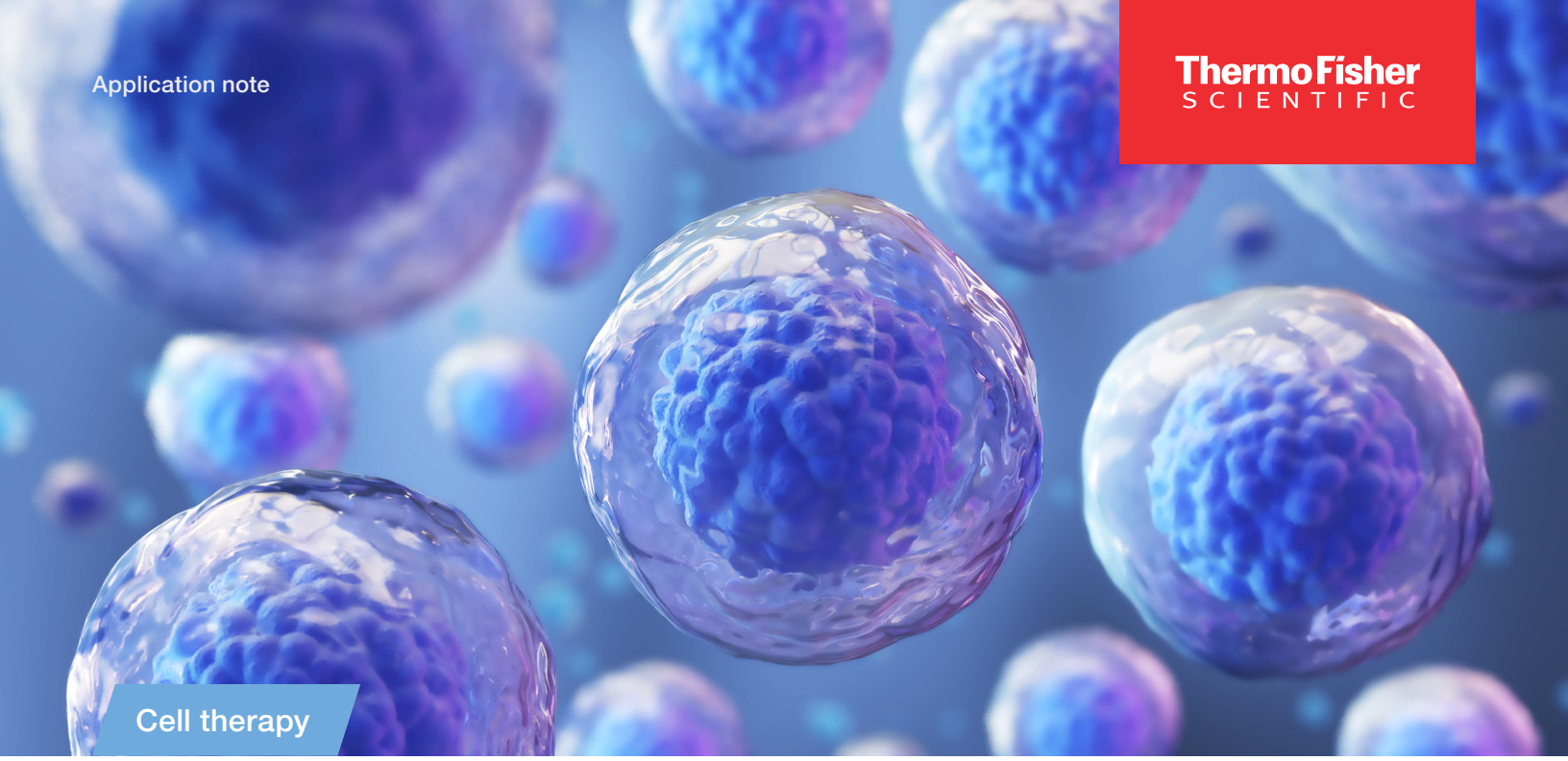
Application note



Cell therapy

Improved T cell expansion using closed, automated stirred-tank bioreactors and CTS OpTmizer Pro SFM

Keywords
Cell therapy, T cell, scale-up, stirred-tank, bioreactor, agitation, perfusion, automation, cell viability, cell density, fold expansion

Introduction

Several T cell therapies have been approved to treat liquid malignancies in patients who do not respond to traditional treatments. In addition to improving the lives of these patients, these therapies possess the potential to address countless other disease states. As such, the T cell therapy market has recently flourished and is expected to grow to $20.3 billion by 2028 [1].

In this rapidly expanding field, automation, improved control, and preparation for effective scale-up are critical to the success of these therapies. This has led cell therapy manufacturers to lean heavily on the use of bioreactors for cell expansion, because of their closed and automated design as well as their ability to create an optimal cell environment.

Rocking motion bioreactors have long been the mainstay in T cell therapy, but stirred-tank bioreactors, such as the Thermo Scientific™ HyPerforma™ Glass Bioreactor, have garnered a lot of excitement because of several potential advantages. These include a larger range of scalability, a smaller footprint, no “dead spots”, and even more intimate control over gassing, feed, and agitation strategies. Gassing can be carried out differently for the various bioreactors, such as through the use of drilled-hole sparges for the stirred-tank bioreactor. This can be useful when headspace is insufficient to maintain dissolved oxygen (DO) or high viable cell density. In addition to gas exchange, stirred-tank reactors allow for fed-batch or perfusion protocols and more thorough liquid mixing with different impeller strategies or varying agitation speeds.

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One important consideration when scaling up to larger bioreactors is the culture medium. Success in smaller-scale settings does not guarantee the same at larger scales, which tend to be associated with high cell densities. Successful workflows at these scales require a very robust culture medium, such as Gibco™ CTS™ OpTmizer™ T Cell Expansion Serum-Free Medium (SFM), no phenol red, and Gibco™ CTS™ OpTmizer™ Pro SFM, both of which have been successfully applied to the consistent cultivation of T cells in rocking motion bioreactors and stirred-tank bioreactors using both fed-batch and perfusion strategies.

Herein we discuss a process that was developed for expanding T cells in a 3 L HyPerforma Glass Bioreactor using CTS OpTmizer Pro SFM and present the results alongside those from several other bioreactors.

Materials and methods

T cell isolation

Primary human T cells from healthy donors were negatively isolated from peripheral blood mononuclear cells (PBMCs) using the Invitrogen™ Dynabeads™ Untouched™ Human T Cells Kit (Cat. No. 11344D). To see T cell isolation materials and methods for process development and clinical use, please see the application note "One-step isolation and activation of naive and early memory T cells with CTS Dynabeads CD3/CD28".

T cell expansion medium

Primary T cells were cultured in CTS OpTmizer Pro SFM (Cat. No. A4966101). This product contains two parts: 1 L basal medium and 26 mL CTS OpTmizer Expansion Supplement. The components were combined per the user manual to create the complete culture medium, which was additionally supplemented with 4 mM Gibco™ GlutaMAX™ Supplement (Cat. No. 35050061) and 2.5% Gibco™ CTS™ Immune Cell Serum Replacement (ICSR; Cat. No. A2596101).

T cell activation (days 0–3)

T cells were seeded at 1 x 10^6 cells/mL in static vessels and activated with Gibco™ Dynabeads™ Human T-Expander CD3/CD28 at a ratio of 3 beads per T cell in the presence of 10 ng/mL of rIL-2, for bag experiments.

Inoculation and run configuration

Multiple stirred-tank bioreactors, a rocking motion bioreactor, and a static control bioreactor were compared to assess fold expansion, viability of cells, and $CD3^+$ cell growth. T cells also were assessed following ramped-up agitation in the stirred-tank bioreactor as well as in perfusion versus fed-batch protocols.

The pH, temperature, dissolved oxygen (DO), and media exchange rates were set to the same levels for each period. However, the precision of the control was higher for the stirred-tank and rocking motion bioreactors. The parameters are shown in Table 1.

Table 1. Parameters for the stirred-tank bioreactor (3 L HyPerforma Glass Bioreactor) and the rocking motion bioreactor (Xuri™ Cell Expansion System).

Parameter	Stirred-tank	Xuri bioreactor
Medium	CTS OpTmizer Pro SFM (basal medium and supplement), 2.5% CTS ICSR, 4 mM glutamine	
Activation/ stimulation	3 Dynabeads CD3/CD28 per T cell (day 0), 10 ng/mL rIL-2 (every 2–3 days)	
Inoculation	Day 3 after activation, 1–2.5 x 10^5 cells/mL	
pH	Maintained above 6.9 (perfusion or base addition)	
Temperature	37°C	
DO	30%	
O_2 delivery method	Drilled-hole L sparge (0–2,000 mL/min) or headspace (0–250 mL/min)	Headspace 0–250 mL/min
CO_2 (Headspace)	6.6% of total gas	
Agitation method	Dual pitched-blade impellor	Wave motion
Agitation setting	120–600 rpm	10–15 rpm, 8–10°
Perfusion strategy	Begin 50% VVD at 2 x 10^6 cells/mL, increase with higher VCD	

Stirred-tank bioreactors

On day 3 after activation, the cells were inoculated into either the 3 L HyPerforma Glass Bioreactor, the BIOne™ Single-Use Bioreactor (Distek), or the BioBLU™ Single-Use Bioreactor (Eppendorf) at a density of 2 x 10^5 cells/mL in 750 mL of expansion medium containing 100 IU/mL of rIL-2. On day 5, the volume of the bioreactors was brought up to 1,200 mL, and 100 IU/mL of rIL-2 was added. On day 7, the volumes were brought up to 2,400 mL, and 100 IU/mL of rIL-2 was added. Up to this point, a fed-batch process was used. Perfusion was initiated when the cells reached 2 x 10^6 cells/mL. Cell growth and viability were monitored daily, and the phenotype was assessed at day 10 using flow cytometry. All data are representative of at least 3 experiments performed on multiple T cell populations from independent healthy donors.

Xuri Cell Expansion System W25 (Cytiva)

On day 3 after activation, the cells were inoculated into 2 L perfusion bags for the Xuri rocker platform at a density of 2 x 10^5 cells/mL in 300 mL of expansion medium containing 100 IU/mL of rIL-2. On day 5, the volume of the vessels was brought up to 500 mL, and 100 IU/mL of rIL-2 was added. On day 7, the volume of the vessels was brought up to 1,000 mL, and 100 IU/mL of rIL-2 was added. Up to this point, a fed-batch process was used. Perfusion was initiated when the cells reached 2 x 10^6 cells/mL. Cell growth and viability were monitored daily, and the phenotype was assessed at day 10 using flow cytometry. All data are representative of at least 3 experiments performed on multiple T cell populations from independent healthy donors.

G-Rex™ cell expansion system (Wilson Wolf)

On day 3, 5 x 10^6 cells were transferred to a static G-Rex™ 6M Well Plate with 100 mL of fresh culture media containing 100 IU/mL of rIL-2. On days 5 and 7, 50 mL of spent medium was exchanged for 50 mL of fresh medium, and rIL-2 was replenished at 100 IU/mL. Cell growth and viability were monitored every 2–3 days, and the phenotype was assessed when indicated using flow cytometry. All data are representative of at least 3 experiments performed on multiple T cell populations from independent healthy donors.

Flow cytometry

Cellular phenotype was assessed on day 10 by staining T cells with Invitrogen™ CD3 Pacific Orange™, CD4 FITC, CD8 Pacific Blue™, CD62L APC, and CD27 PE antibodies and analyzed using the Invitrogen™ Attune™ NxT Flow Cytometer.

Results

Bioreactor comparison

The HyPerforma stirred-tank bioreactor, Xuri rocking motion bioreactor, and G-Rex static control bioreactor were compared to assess fold expansion, viability of cells, and $CD3^+$ cell growth. These results are representative of at least 3 independent experiments.

Overall, dynamic reactors, i.e., rocking motion or stirred-tank reactors, achieved higher-fold expansions, with the stirred-tank consistently producing the highest. In the example presented here, with one particular donor, the cell culture in the stirred-tank bioreactor had a fold expansion of approximately 300 at day 10, while the rocking motion bioreactor and the static control bioreactor reached ~225 and ~150, respectively (Figure 1). The stirred-tank and rocking motion bioreactors had ~95% viability, compared to a lower viability for the static bioreactor at day 10 (Figure 2).

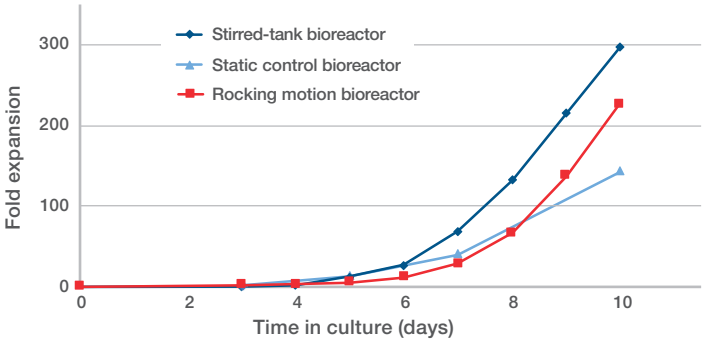

Figure 1. Fold expansion comparison of T cells in three different bioreactors. Increased fold expansion was achieved in the stirred-tank bioreactor, followed by the rocking motion bioreactors and static bioreactors.

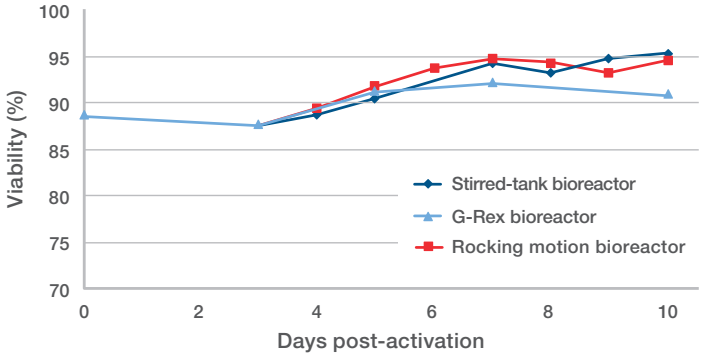

Figure 2. Viability of T cells in three different bioreactors. The stirred-tank and rocker bioreactors had ~95% viability, compared to a lower viability for the static bioreactor at day 10.

All three bioreactors provided a desirable phenotype distribution, with a high number of early memory cells and low effector numbers. There was no significant difference in cell phenotype between bioreactors (Figure 3).

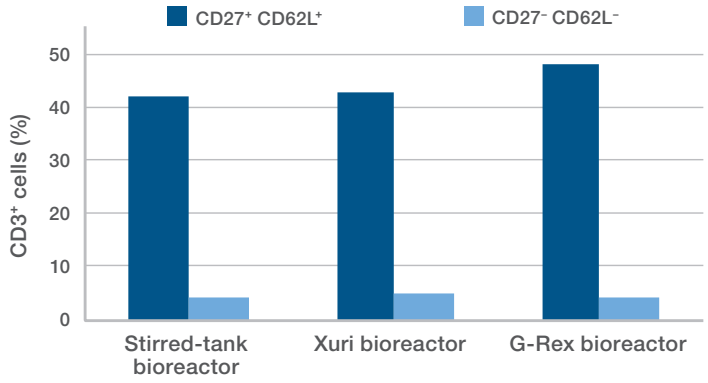

Figure 3. Differentiation of T cells in different bioreactors. Phenotype comparison between the cultures showed that all three bioreactors provided a desirable phenotype distribution, with a high number of early memory cells and low effector numbers.

Increased agitation

The two fed-batch cultures grown in stirred-tank bioreactors were compared. One had an agitation ramp up between 120 and 600 rpm, and the other one employed a steady-state agitation of 120 rpm. Ramping up agitation improved fold expansion by ~20%, as shown in Figure 4. The culture without ramped-up agitation plateaued at a fold expansion of ~250, while the ramped agitation culture expanded beyond 300. There was also a small but consistently observed benefit in viability with ramped-up agitation (Figure 5). There was no significant change in differentiation state between the two conditions, with both showing low levels of effector cells (data not shown).

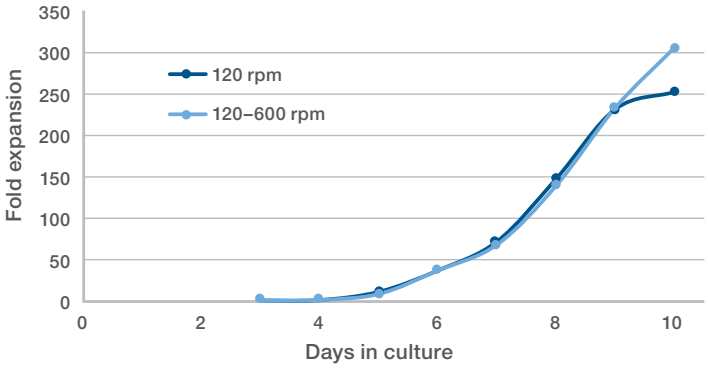

Figure 4. Fed-batch cultures grown in stirred-tank bioreactors with differing agitation. Ramping agitation between 120 rpm and 600 rpm improved fold expansion by ~20%.

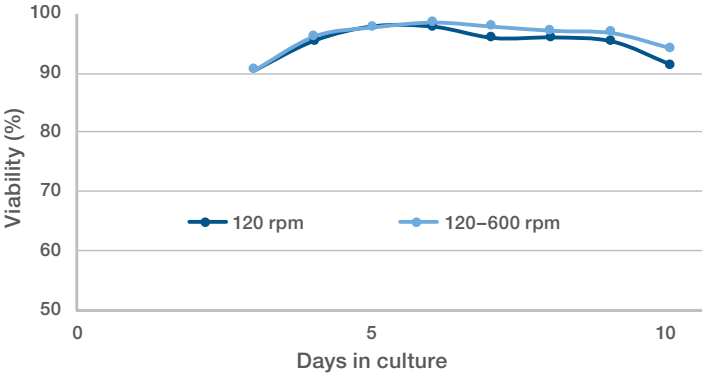

Figure 5. Comparison of cell viability between two fed-batch cell lines in stirred-tank bioreactors. Ramped agitation results in a consistent benefit in cell viability.

The steady-state and ramped-up cultures were then compared against cultures grown in two alternative single-use stirred tanks, the BioBLU and BIOne bioreactors. For steady agitation, regardless of bioreactor and growth trajectory, cultures peaked at approximately 10–12 x 10^6 cells/mL (Figure 6). Ramping up agitation allowed for higher viable cell density, reaching 16 x 10^6 cells/mL with no effect on differentiation.

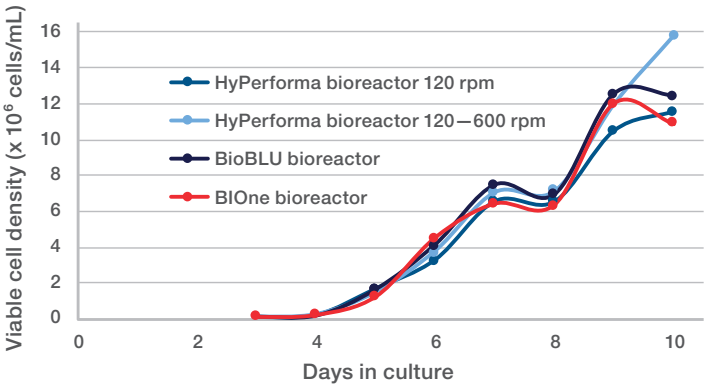

Figure 6. Comparison of viable cell densities between different stirred-tank bioreactors and agitation conditions. At steady agitation, regardless of vessel, cultures peaked at approximately 10–12 x 10^6 cells/mL. Ramping agitation allowed for higher viable cell density, with no effect on differentiation.

Fed-batch versus perfusion

The growth of perfusion and fed-batch cultures were compared in stirred-tank bioreactors. When comparing fold expansion, a divergence occurred around day 6 or 7 between perfusion and fed-batch cultures. Perfusion appeared to help sustain more growth (Figure 7). The fed-batch culture plateaued at around 10 x 10^6 cells/mL at day 10, while perfusion reached 20 x 10^6 cells/mL in the same amount of time. This growth for the perfusion culture continued for approximately 2 days, reaching an even higher cell density. Perfusion also helped to maintain higher viabilities (Figure 8). At high cell densities, perfusion maintained over 95% viability, while the fed-batch culture started to fall below 90% around day 9.

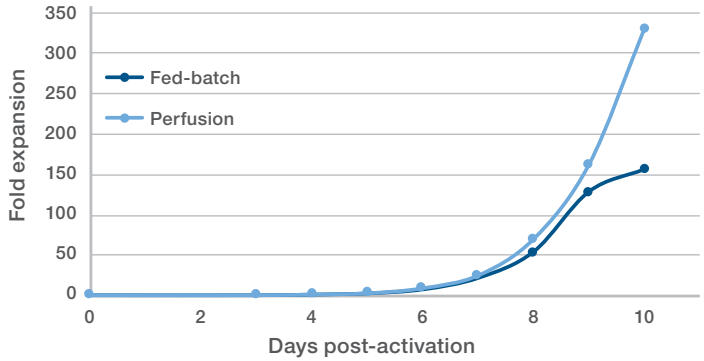

Figure 7. Fold expansion in a fed-batch culture and a perfusion culture. Perfusion resulted in more cell growth, whereas the fed-batch culture began to plateau at day 10.

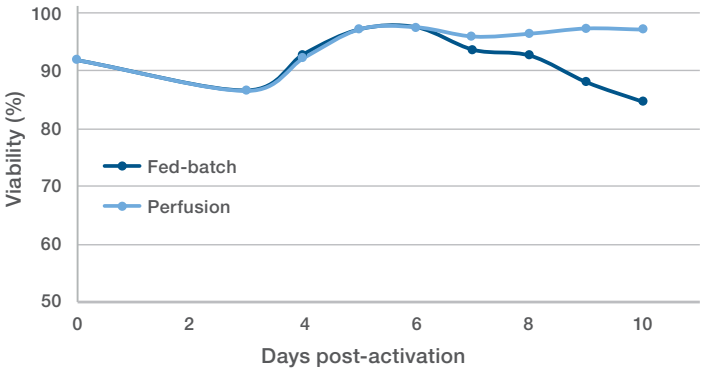

Figure 8. Viabilities of fed-batch and perfusion cultures. At high cell densities, perfusion maintained over 95% viability, while the fed-batch culture started to fall below 90% around day 9.

There was little difference in the phenotype between the perfusion and fed-batch cultures, apart from a small increase of effector cells in the perfusion culture (Figure 9). The same percentage of early memory cells was seen.

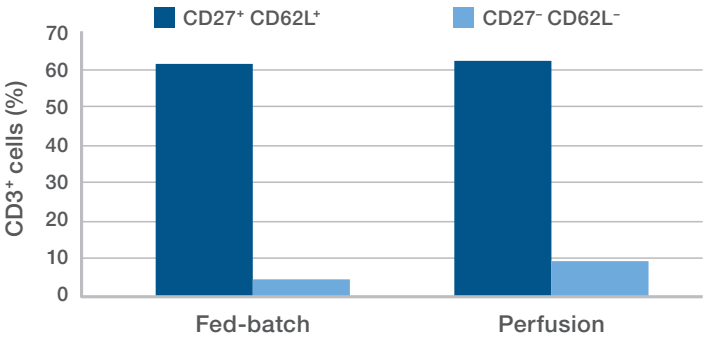

Figure 9. Phenotype between fed-batch and perfusion cultures. There was little difference in the phenotype between the fed-batch and perfusion cultures.

Discussion and conclusion

T cell therapies have already demonstrated success in liquid malignancies. These early wins have led researchers to increase efforts toward developing process innovations that will further improve the efficacy and safety of these therapies. This could potentially allow for both the treatment of solid tumors and the production of allogeneic cell therapies. As such, enhanced automation capabilities and process controls in these closed and automated systems are a point of emphasis. Stirred-tank bioreactors have long been a staple in bioprocesses for mature modalities, in large part because of their excellent flexibility and control. It is these same qualities that make them excellent candidates to be used in the expansion of cell therapies.

The data presented provide evidence that dynamic reactors are better for T cell growth and expansion than static platforms. In these experiments, stirred-tank bioreactors performed better than rocking motion bioreactors. This correlates with the increased control capabilities each one provides. These investigations also suggested that ramping up agitation had a positive impact on T cell growth and density while retaining phenotype, regardless of perceived levels of cell stress. Perfusion also had a positive impact on cell expansion and viability of T cells. These results therefore highlight a potential methodology that can deliver high levels of performance.

Finally, these results were produced at stirred-tank volumes ranging from 700 mL to 2.4 L, but it is important to note that stirred-tank bioreactors boast excellent scalability. This means that processes like those described here could be an option for a wide range of applications, including autologous and allogeneic workflows.

Based on the above data and increased environmental control of stirred-tank bioreactors, they are ideal for T cell therapy manufacturing, where more control correlates with improved performance. Combining this methodology with a robust and specialized medium like CTS OpTmizer Pro SFM could help to provide more control and enhance the scale-up process, as you move toward clinical and commercial manufacturing.

Reference

1. T-cell therapy market size, share & trends analysis report by modality, by therapy (CAR T-cell, tumor-infiltrating lymphocytes), by indication (hematologic malignancies, solid tumors), by region, and segment forecasts, 2021–2018, https://www.grandviewresearch.com/industry-analysis/t-cell-therapy-market.

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