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Application Note

October 1, 2020

Keywords or phrases:
CAR-T viability, CAR-T yield, inline monitoring, process control, single-use, consistent T-cell media, serum-free T-cell media

CAR-T Expansion Using the Sartorius T-Cell Expansion Solution

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Abstract

Historically, process development scientists have repurposed bioprocess technologies equipment for cell-based therapies due to the lack of fit-for-purpose hardware and consumables for cultivating immune cells. The ability to monitor and control processes through online sensors as well as the availability of consistent media and high-quality single-use consumables for the expansion of T-cells are critical customer needs. It is imperative that tool providers address these needs when developing solutions to enable cellular immunotherapy manufacturers to bring their cutting-edge therapies to market faster.

The Sartorius T-Cell Expansion Solution, which consists of the Biostat® RM TX bioreactor, Flexsafe® RM TX bag, and 4Cell® Nutri-T medium, simplifies T-cell process development and manufacturing by enabling process control and consistency. This functionally closed system with automated online process controls for pH, DO, and viable biomass works seamlessly with the single-use, optimized culture bag and the consistent, specifically designed and optimized T-cell medium. This application note demonstrates the successful expansion with inline monitoring of chimeric antigen receptor (CAR) T-cells from two donors using the Sartorius T-Cell Expansion Solution.

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Introduction

CAR-T immunotherapy continues to show great promise for the treatment of some of the most aggressive cancers. Since 2017, three CAR T-cell-based therapies have been approved, including Kymriah™ for B-cell precursor acute lymphoblastic leukemia in 2017 and for relapsed or refractory large B-cell lymphoma in 2018; Yescarta™ for relapsed or refractory large B-cell lymphoma in 2017; and Tecartus™ for relapsed or refractory mantle cell lymphoma in 2020.

As the demand for personalized therapy continues to grow, so does the development and commercialization of CAR-T, with over 100 companies now reported to be either developing CAR-T therapies, acquiring companies that are developing CAR-T therapies, or partnering with companies to develop CAR-T therapies.[1] Developers and manufacturers must both optimize and efficiently accelerate production, which depends on a consistent supply of raw materials.[2] Further, the consistency and affordability of immunotherapy is limited by high manufacturing costs.

Here we demonstrate the use of the purpose-built Sartorius T-Cell Expansion Solution to address manufacturing challenges of autologous CAR-T expansion. This automated, closed system consists of the Sartorius Biostat® RM TX Twin system bioreactor, Flexsafe® RM TX bag, and 4Cell® Nutri-T medium and enables cost-effective, efficient, consistent T-cell expansion (Fig. 1). The low-shear bioreactor offers automated online controls, the single-use bags feature an optimized film and an integrity pre-tested filter, while the 4Cell® Nutri-T medium was specifically developed to promote T-cell expansion without the addition of serum. In the case study that follows, this solution was used for the successful expansion and online monitoring of CAR T-cells.

Materials and Methods

Materials

- Biostat® B Twin controller unit
- Biostat® RM TX Twin system
- Flexsafe® RM TX bags (2L) with perfusion membrane and single-use pH and DO sensors
- 4Cell® Nutri-T medium
- iQue® ScreenerPlus

Cells

Human primary T-cells were enriched from cryopreserved PBMC (Lonza) obtained from two healthy donors (donor 1, donor 2). PBMC were thawed and seeded at a density of 2×10^6 cells/mL in T-cell culture medium (4Cell® Nutri-T medium supplemented with 600 U/mL IL-2 (Miltenyi Biotec)) in static T flasks (Greiner Bio-One) at 37°C in a humidified 5% CO2 incubator. After an overnight rest, cells were transferred to new T flasks and activated at a density of 1×10^6 cells/mL using 1:100 TransActTM (Miltenyi Biotec).

Transduction

After 24h of activation, T-cells were transduced using third generation, VSV-G pseudotyped lentiviral vectors (internal production) coding for the anti-CD19 CAR and a truncated form of the epidermal growth factor receptor (EGFRt). EGFRt was used as a transduction marker. Transduction was performed using a MOI of 2 and 4 µg/ml polybrene. Two days after lentiviral infection, the virus-containing medium was removed and replaced with T-cell culture medium.

CAR T-cell expansion in the Biostat® RM TX Twin system

Cell expansion using cells from two donors was performed in parallel using the Biostat® RM TX Twin system (Sartorius) together with Flexsafe® RM TX single-use 2L bags (Sartorius) equipped with an integrated 1.2 µm perfusion membrane and single-use pH and DO sensors.

Fig. 1: Sartorius T Cell Expansion Solution consisting of the Biostat® RM TX Twin bioreactor system, Flexsafe® RM TX bag, and 4Cell® Nutri-T medium.

To generate the CAR T-cell seed train for RM TX inoculation, cells were thawed 7 days before inoculation, activated, transduced, and cultured in T flasks as noted above. Cells were inoculated at a concentration of 0.5×10^6 cells/mL in 0.3 L 4Cell® Nutri-T medium supplemented with 600 U/mL IL-2 using the parameters outlined in Table 1. On culture days 1 and 2, the culture volume was increased to 0.6 L and 1 L, respectively, via the addition of 4Cell® Nutri-T medium. Starting on day 3, perfusion with 4Cell® Nutri-T medium was performed (600 mL/d) until the end of culture (day 5).

Parameter	Set Point
CO_2	2 ccm
Air	38 ccm
Rocks	6 rpm, increased to 10 rpm during perfusion
Angle	6°
pH	Monitored
DO	Monitored
Temperature	37°C

Table 1: Biostat® RM TX process parameters.

Determination of transduced cells

Transduced T-cells were determined by flow cytometry. Cells were suspended in phosphate-buffered saline (PBS, Carl Roth) stained for 10 min at room temperature using the viability dye Zombie NIR™ (Biolegend), followed by 15 min fixation using 5% Roti® Histofix (Carl Roth). Cells were washed with staining buffer containing 1% bovine serum albumin (BSA, Carl Roth) and stained for 20 min at room temperature using anti-human EGFR phycoerythrin (PE)-conjugated antibody (R&D Systems). Cells were washed twice in staining buffer and analyzed using the Sartorius iQue® ScreenerPlus platform, a flow cytometry instrument which enables high-content, multiplexed analysis of cells in suspension, along with the integrated ForeCyt® software. As a negative control, non-transduced cells were stained using the same protocol.

Cell count & viability measurements

Cell count and viability measurements were performed based on the trypan blue exclusion method using the CEDEX HiRes Analyzer (Roche).

Results

Monitoring of CAR-T expansion

This case study presents results of CAR T-cell expansion in the Biostat® RM TX Twin system using Flexsafe® RM TX bags (2L) with perfusion membrane and 4Cell® Nutri-T media. To directly measure donor-to-donor variability, cells obtained from two healthy donors were cultured in parallel using identical process parameters and the same media and reagent batches. Prior to bioreactor expansion, cells were thawed, transduced, and pre-cultured in static T-flasks to reach sufficient cell numbers for bioreactor inoculation (data not shown). During bioreactor expansion, 4Cell® Nutri-T media supplemented with IL-2 was added daily to obtain a cell concentration in the range of $0.4 \times 10^6 - 0.6 \times 10^6$ cells/mL until the maximum culture volume of 1L was reached on day 3. From then on, perfusion was used.

Cells were counted daily and similar growth curves for both donors were observed (Fig. 2). Cells showed exponential cell growth until the culture was terminated on day 5, reaching a total cell count of $7.7 \times 10^9 - 7.8 \times 10^9$ cells in 1 L culture volume.

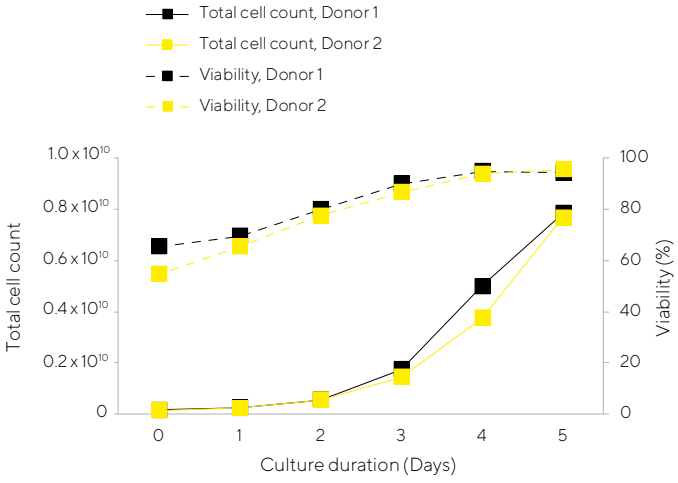

Fig. 2: Excellent cell growth and improvement in cell viability using the Sartorius T-Cell Expansion Solution. Cells from two healthy donors were expanded for 5 days in Flexsafe® RM TX bags using the Biostat® RM TX Twin system using daily media feeds (day 1 and day 2) or perfusion (day 3 and on) with 4Cell® Nutri-T media supplemented with 600 U/mL IL-2. Cell count and viability were determined using offline measurements. Indicative of successful adaptation, cell viability improved from 55-65% to >90% during expansion using the Sartorius T-Cell Expansion Solution.

Cell viability at the time of inoculation was relatively low (55% - 65% for donor 1 and 2, respectively) due to low viability of the cell inoculum obtained from static pre-culture (data not shown). However, viability of both donors

increased to >90% during culturing in the bioreactor, suggesting that the cells not only adapted to the rocking motion bioreactor conditions, but the applied process parameters and feeding regime enabled the recovery of cell viability.

In addition to monitoring total cell growth, the increase of CAR T-cells was determined. For this, the number of CAR T-cells at inoculation (day 0) and at the end of culture (day 5) was calculated (Fig. 3). While the total number of CAR T-cells increased for both donors, donor 1 showed a notably higher number of CAR-positive cells, both at inoculation and on day 5, resulting in a yield of 1.5×10^8 CAR T-cells for donor 1 compared to 5.2×10^7 CAR T-cells for donor 2. This is likely due to the higher transduction efficiency obtained for donor 1, resulting in an overall higher percentage of CAR-positive cells in the cultured cell population.

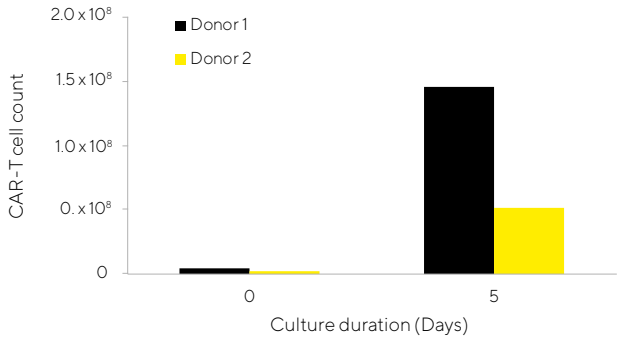

Fig. 3: Successful expansion of CAR T-cells using the Sartorius T-Cell Expansion Solution. The total number of CAR-positive T-cells was determined at inoculation (day 0) and at the end of culture (day 5). Donor differences likely represent different transduction efficiencies.

Online monitoring of pH and DO

For this case study, pH and DO were not regulated during cell expansion in the bioreactor. Instead, a constant gas flow consisting of 5% CO_2 and 95% air was applied to mimic a standard CO_2 incubator environment. However, to evaluate the behavior of pH and DO throughout the duration of the culture, pH and DO were monitored online using the optical, single-use pH and DO sensors integrated in the Flexsafe® RM TX single-use 2 L bags. Both parameters showed similar behavior for the two donors (Fig. 4). pH decreased slowly but steadily from 7.6 after inoculation and leveled out between 7.2 – 7.3 towards the end of culture. For DO, only a slight decrease was observed until day 2, suggesting that the daily medium feeds performed in fed-batch mode during this time were sufficient to keep DO at a fairly constant level. After perfusion was started (day 3), DO started to decrease remarkably, indicating that the perfused fresh medium was not able to prevent a decrease in DO, especially as the cell concentration increased exponentially at the same time.

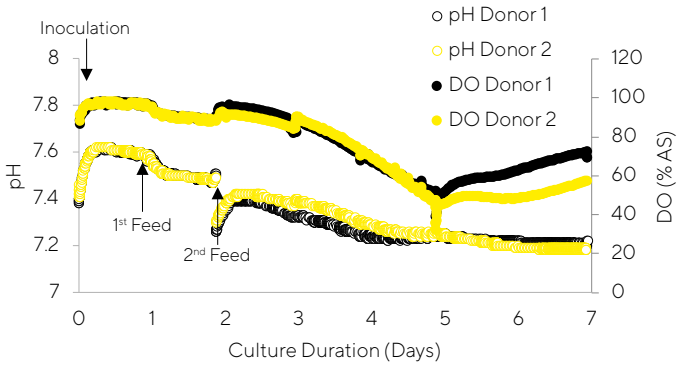

Fig. 4: Online monitoring of pH and DO in the donor cultures. Instead of pH and DO regulation during expansion, a constant gas flow consisting of 5% CO_2 and 95% air was applied. Despite the lack of active regulation, this expansion using the Sartorius T-Cell Expansion Solution yielded both high cell counts and increased viability for both donors.

In summary, pH and DO decreased as expected due to the fact that neither parameter was regulated. Interestingly, an increase in cell count and viability was maintained at the same time, suggesting that the applied process parameters are sufficient to support cell growth.

Discussion

The case study above illustrates the utility of the Sartorius T-Cell Expansion Solution to successfully expand and monitor CAR-T from two individual donors. Despite the low cell viability (55-65%) of the initial inoculum for both donors, cell viability was recovered upon expansion (>90%). The integrated optical, single-use pH and DO sensors enabled continuous monitoring of pH and DO throughout the course of the expansion. Despite the observed decrease in pH and DO, however, cell count increased and viability was maintained. A next step in the optimization would be engagement of the built-in automated process controls to maintain constant pH and DO.

The use of the Sartorius T-Cell Expansion Solution represents a powerful, integrated solution that is well-suited to address today's manufacturing challenges for consistent, high-yield CAR-T expansion. The Biostat® RM TX system supports the transition from process development to manufacturing under regulatory compliance. The optimized 4Cell® Nutri-T medium enables excellent CAR T-cell expansion and viability.

Conclusion

As demonstrated here, the Sartorius T-Cell Expansion Solution is an automated, integrated, simplified solution for many of the challenges in CAR T-cell expansion process development. The CAR-T cultures expanded in this functionally closed system with low E&L-profile bag and consistent serum-free medium showed high yields and dramatically increased viability. The T-cell yield was clinically relevant (>1 x 10^8). Further optimization, such as implementation of the built-in automated online controls, can improve yield while maintaining high cell viability and will be investigated in future studies. The use of the Sartorius T-Cell Expansion Solution may lead to faster and more cost-effective CAR-T therapies, creating a much-needed path to the development of more affordable and timely treatments for patients.

Acknowledgements

The authors would like to thank Katja Heinrichs and Sinan Oender for excellent experimental support.

References

1. Hildreth, C. (2020, September 8th). CAR-T companies proliferate: list of CAR-T companies Worldwide. BioInformant. https://bioinformant.com/car-t-companies-the-meteoric-rise-of-cellular-immunotherapie
2. Papathanasiou MM, Stamatis C, Lakelin M, Farid S, Titchener-Hooker N, Shah N. Autologous CAR T-cell therapies supply chain: challenges and opportunities? [published online ahead of print, 2020 Jan 14]. Cancer Gene Ther. 2020;10.1038/s41417-019-0157-z. doi:10.1038/s41417-019-0157-z

Ordering information

Description	Part Number
Biostat® RM TX 2L Single, basic	1903-FAC-5026
Biostat® RM TX 2L Twin, basic	1903-FAC-5027
Biostat® B Twin	FERM-B2EVAL-17
Biostat® B Single	FERM-B1EVAL-17
BioPAT® ViaMass SU	BPV0001
Flexsafe® RM TX 2L perf. ViaMass, TPE (3 pcs)	DFT002L-SMVM1
Flexsafe® RM TX 2L perf. ViaMass, PVC (3 pcs)	DFT002L-SMVM2
4Cell® Nutri-T Medium, 1L	05-11F2001-1K

Recent publication

Hupfeld J, Henrichs K, Kraft M, and Tappe, M. Comparison of primary T cell cultured in static conditions versus rocking motion bioreactors. Cytotherapy, May 2020, Volume 22 (5), Supplement S147 https://doi.org/10.1016/j.jcyt.2020.03.298

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DIR: 2475299-000-02
Status: 02 | 2020