New perfusion filter demonstrates improved cell retention when expanding human T cells in Xuri™ Cell Expansion System

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Introduction

Xuri™ brand Perfusion Cellbag™ bioreactors (previously known as WAVE™ Bioprocess Cellbag bioreactors) are regularly used for *ex vivo* T cell expansion in chimeric antigen receptor (CAR) T cell therapy manufacturing. The WAVE™ brand Cellbag™ bioreactor, with a filter membrane of 7–12 µm average pore size, was designed to expand cells used in bioprocess applications. Because T cells are 7–13 µm on average, using these bags for perfusion-based T cell expansion resulted in cell loss, decreased cell retention, and inability for further expansion to the desired cell density. Therefore, a new perfusion filter membrane was developed specifically for T cell expansion. The new 1.2 µm pore size filter was validated for use in the Xuri™ Cell Expansion System W25, which consists of a reusable instrument and single-use Cellbag™ bioreactors. The substantially smaller pore size of the new filter membrane decreased T cell loss, ultimately leading to higher T cell retention in the Xuri™ brand Cellbag™ bioreactor. These results can correlate to attaining the dosing density of a given cell therapy quicker compared to using a larger pore size filter, which could decrease the processing time for cell therapy manufacturers. A decreased processing time could also reduce the cost of the therapy.

Materials and Methods

Activation of T cells in static culture

Frozen human peripheral blood mononuclear cells (PBMCs) were thawed, washed twice with Xuri™ T Cell Expansion Medium (Cytiva) supplemented with 5% heat-inactivated human serum (Akron, Gemini), 1% penicillin-streptomycin (Gibco™) and 350 IU/mL of IL-2 (GE, Peprotech) and cultured in T225 flasks at 1 × 10E6 cells per mL. CTS Dynabeads™ CD3/CD28 beads (Life Technologies) were added to the culture at a ratio of 3:1 beads: CD3+ T cells. After 3 days of static conditions (37°C, 5% CO2, 95% RH), cells were counted and maintained at 1 × 10E6 cells per mL via batch feeding until they reached the required cell number. Then, cells were transferred into Xuri™ brand Cellbag™ bioreactors at 5 × 10E5 cells/mL final density using Xuri™ Cell Expansion System W25, Xuri™ System W5, or WAVE™ Bioreactor 2/10 System.

Perfusion strategy

To test the rigor and robustness of the new filter material, a perfusion schedule was created to allow perfusion of high and consistent volumes across all Cellbag™ bioreactors. A semi-continuous perfusion strategy, considered the worst-case scenario, would provide confidence in the integrity and function of the new filter. Two Xuri™ brand Cellbag™ sizes (2 and 10 L) were evaluated. The rocking rates and angles, and perfusion schedules are shown in Tables 1 and 2, respectively. The waste bag was exchanged daily and waste volume measured to determine daily cell loss. Samples were taken daily from each Cellbag™ bioreactor and waste bag to assess cell count, cell size, and viability.

Phenotypic analysis

Phenotypic analysis was performed on cells cultured in Cellbag™ bioreactors constructed with either the current filter or new 1.2 µm filter assembly. The cells were cryopreserved on the final day of expansion and immunophenotyped using flow cytometry. Cells (1×10E6) were stained with CD3-perCPCy™5.5, CD4- PE and CD8-Alexa Fluor™ 488. Cells were analyzed with a BD LSRFortessa™ flow cytometer using FACSDiva™ software, according to the manufacturer's instructions (reagents, instrument, and software from BD Biosciences). FlowJo™ software (FlowJo™, LLC) was used for data analysis.

Results

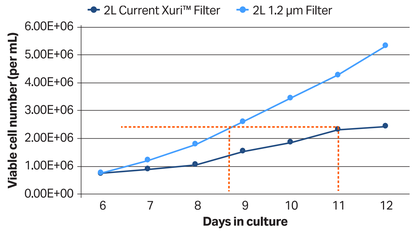

Fig 1. A representative growth curve for 1 L expansion of T cells cultured in Cellbag™ bioreactors with the new 1.2 µm perfusion filter or filter in current Xuri™ Cellbag™ ioreactobr. Data represents the mean and SD of the daily cell counts, n = 3 (SD too low to be visible). The graph illustrates the benefit of cell retention on the total cell yield and time required to achieve a defined cell number. The new 1.2 µm filter has the potential to reduce the time to achieve a predefined cell number and to increase cell yield.

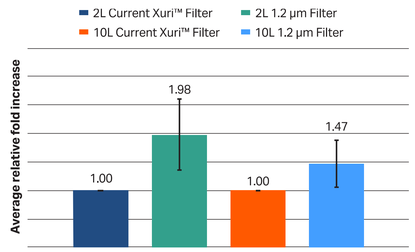

Fig 2. T cell expansion data comparing the new 1.2 µm perfusion filter and current filter in 2 L and 10 L Cellbag™ bioreactors. Cell growth was analyzed using total relative fold increase after the final day of expansion. Relative fold increase is calculated by dividing the fold increase of the Cellbag™ bioreactor with 1.2 µm filter by the fold increase of the bioreactor with current filter. Relative fold increase is used to standardize analysis across all runs. Data represents the mean and SD. 2 L current filter n = 4; 2 L 1.2 µm filter n = 15; 10 L current filter n = 3; 10 L 1.2 µm filter n = 11.

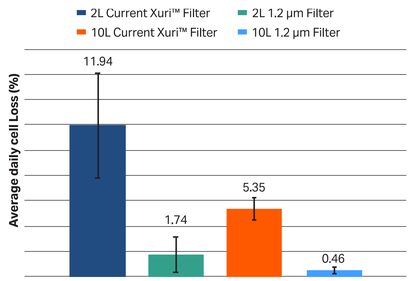

Fig 3. Cell loss was computed as total average daily cell loss over the course of the expansion. This reduction in cell loss was observed across both 2 L and 10 L Cellbag™ bioreactors constructed with the new 1.2 µm filter assembly. Data represents the mean and SD. 2 L current filter n = 4; 2 L 1.2 µm filter n = 15; 10 L current filter n = 3; 10 L 1.2 µm filter n = 11.

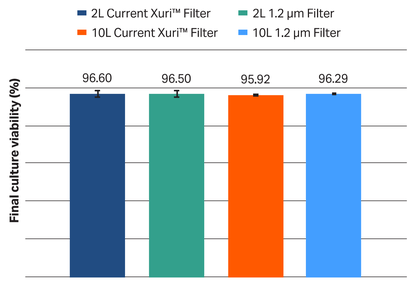

Fig 4. Cell viability was assessed on the final day of expansion to confirm that the new 1.2 µm filter did not have a negative impact on cell health. No obvious differences in cell viability were observed across both filter assemblies and Cellbag™ bioreactor sizes. Data represents the mean and SD. 2 L current filter n = 4; 2 L 1.2 µm filter n = 15; 10 L current filter n = 3; 10 L 1.2 µm filter n = 11.

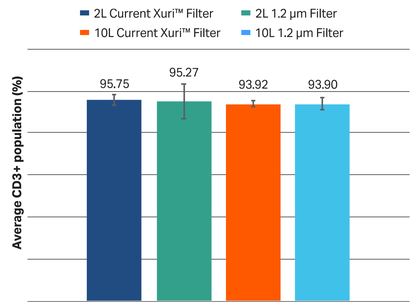

Fig 5. Phenotypic analysis shows the consistent population of CD3+ across both filter assemblies and bioreactor sizes. Data represents the mean and SD. 2 L current filter n = 4; 2 L 1.2 µm filter n = 15; 10 L current filter n = 3; 10 L 1.2 µm filter n = 11.

Conclusions

Table 1. Rocking parameters for both the 2-liter and 10-liter Xuri™ Cellbag™ bioreactors

Perfusion day	Rocks/ min	Angle °
1	10	6
2	10	6
3	12	6
4	12	6
5	15	6
6	15	6

Table 2. Perfusion strategy for 2-liter and 10-liter Xuri™ Cellbag™ bioreactors

2 L PERFUSION STRATEGY

Perfusion day	Perfusion volume (total)	Shot volume	Shots/ day
1	500	50	10
2	500	50	10
3	1000	50	20
4	1000	50	20
5	2000	50	40
6	2000	50	40

10 L PERFUSION STRATEGY

Perfusion day	Perfusion volume (total)	Shot volume	Shots/day
1	1000	50	20
2	1000	50	20
3	2000	50	40
4	2000	50	40
5	3500	50	70
6	3500	50	70

Conclusions

The improved 1.2 µm filter assembly demonstrated:

- A consistent reduction in cell loss across all runs and Cellbag™ bioreactor sizes
- No impact to cell health as assessed by cell viability
- Similar numbers of CD3+ T cells post-expansion
- Increased cell retention throughout expansion across both Cellbag™ bioreactor sizes
- Faster processing times to reach desired cell density

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This poster was presented at the 2018 Biomedical Engineering Society (BMES): Advanced Biomanufacturing Special Interest Group Conference, August 22, 2018, Worcester, Massachusetts.

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CY26100-31Dec21-PT