Cell therapy

Closed-system media formulations and culture systems for cell therapy manufacturing

Flexible media and reagents for varied and complex workflows

Introduction

The therapeutic value of cell therapy (CT) has been firmly established. There are over 1,000 experimental CT drugs in the approval pipeline, more than half of which are being evaluated in phase 2 clinical trials [1]. However, complex manufacturing processes and the inherent variability of biological materials contribute to significant lot-to-lot variability and high costs. First-generation chimeric antigen receptor T (CAR T) cell manufacturing processes were extremely complicated and labor-intensive, and manufacturing failure rates were often high. The industry has been devising strategies for improving the CT manufacturing process to ultimately reduce variability, risk, and cost. One of these strategies is moving towards closed and automated manufacturing systems.

There are many closed and automated manufacturing options for cell expansion workflows, including rocking and stirred-tank bioreactors. Stirred-tank bioreactors have been widely adopted for production of therapeutic biologics, and CT developers are recognizing their potential advantages for smaller-volume autologous and large-volume allogeneic CT applications. A stirred-tank bioreactor allows the user more control over gassing, feeding, and agitation, which can improve expansion yield and efficiency. See our application note on **improving T cell expansion using closed and automatic stirred-tank bioreactors** to learn more about bioreactor expansion.

While there have been important advances in developing standardized automated and closed manufacturing systems, many CT manufacturing workflows still require the use of unique media formulations with different components in varying concentrations. In addition to being inconvenient, manually adding media components in a CT manufacturing workflow carries the risk of contamination due to open manual touchpoints.

Tips and tricks for sterile welding

High-quality sterile welding is critical for maintaining sterility when a Thermo Scientific™ BioProcess container (BPC) is connected to a closed system. Remember the following points to help ensure the integrity of your welds:

- Always use tubing that is compatible with your welder. Not all tube dimensions and materials are compatible with all sterile welders.
- Not all pinch clamps work with all bags and tubing lines. Pay close attention to your pinch clamps and ensure all tubes are clamped and unclamped properly.
- Avoid stretching your tubes, as stretching can compromise their integrity and result in leakage.
- Check the integrity of your welds before opening any pinch clamps or connecting tubes to your equipment.

Gibco™ Cell Therapy Systems™ (CTS™) OpTmizer™ Pro SFM (no phenol red) and Gibco™ CTS™ Immune Cell Serum Replacement (SR) are serum-free products developed for the growth and expansion of human T lymphocytes for cell therapy. CTS OpTmizer Pro SFM improves central memory phenotype and cell growth by shifting cellular metabolism. T cells retain their functionality, and a larger population of central memory cells can be obtained in less time. CTS Immune Cell SR is a basal medium supplement that provides additional nutrients, and it can reduce the risk and cost associated with using human serum.

Media and supplements packaged in BPCs can help make using complex formulations in CT workflows more convenient. They eliminate the need to transfer media and components to bags or transfer vessels, which greatly reduces the risk of contamination. Perfusion bioreactors provide the additional advantage of allowing individual components to be delivered directly to the bioreactor vessels in specific ratios. In this application note, we compare the performance of open and closed media formulations in rocking and stirred-tank perfusion bioreactors. We demonstrate how integrating off-the-shelf bagged media formulations into a perfusion bioreactor manufacturing process can enable clinically relevant cell expansion to generate therapeutic T cells with fewer manual steps.

Materials and methods

T cell isolation and activation

Primary human T cells obtained from healthy donors were negatively isolated from peripheral blood mononuclear cells (PBMCs) using the Invitrogen™ Dynabeads™ Untouched™ Human T Cells kit (Cat. No. 11344D). For more details about T cell isolation, see our application note on **one-step isolation and activation of naïve and early memory T cells**. The isolated T cells were seeded at 1 x 10^6 cells/mL in static G-Rex™ vessels and activated with Gibco™ Dynabeads™ Human T-Expander CD3/CD28 beads at a ratio of three beads per T cell in the presence of 10 ng/mL interleukin 2 (IL-2) for two days.

T cell expansion medium

The primary T cells were cultured in CTS OpTmizer Pro SFM. CTS OpTmizer Pro SFM is a medium kit that includes Gibco™ CTS™ OpTmizer™ Pro Basal Medium and Gibco™ CTS™ OpTmizer™ T Cell Expansion Supplement. The basal medium and expansion supplement were combined, then supplemented with Gibco™ L-Glutamine (Cat. No. A2916801), Gibco™ CTS™ GlutaMAX™ Supplement (Cat. No. A4737001), and CTS Immune Cell SR (Cat. No. A2596101). Interleukin 2 (IL-2) was added to the complete medium at 10 ng/mL just before the medium was delivered to the bioreactors. The composition of the final medium is shown in Table 1.

Open-system medium preparation

Transfer bottles were fitted with tubing that was compatible with the bioprocess controller for the 3 L Thermo Scientific™ HyPerforma™ Glass Bioreactor and the peristaltic pump of the Xuri™ Cell Expansion System W25 rocking motion bioreactor. The required amounts of the components listed in Table 1 were manually added to the transfer bottles. The complete medium was then perfused into the culture vessels.

Closed-system medium preparation

Tubing used to deliver each medium component to the HyPerforma Glass Bioreactor was sterile-welded to a common feed line. The other ends of the tubes were attached to separate peristaltic pumps on the Thermo Scientific™ HyPerforma™ G3Lab Controller. The flow rates were set to deliver specific quantities of the medium components to prepare the final closed medium formulation (Table 1). The common feed line was connected to the HyPerforma G3Lab Controller for direct perfusion into the culture vessel.

To prepare the medium for the rocking motion bioreactor, a BPC containing CTS OpTmizer T Cell Expansion Supplement was welded to a BPC containing CTS OpTmizer Pro Basal Medium (Table 1). The appropriate amount of each additional supplement was weighed on a calibrated scale and added to the BPC containing the basal medium. The BPC containing the final formulation was sterile-welded to the culture vessel, and the medium was perfused into the culture vessel using the HyPerforma G3Lab Controller.

Table 1. Composition of the final culture medium.

Component	Amount
L-Glutamine	2 mM
Interleukin 2 (IL-2)	10 ng/mL
CTS GlutaMAX Supplement	4 mM
CTS Immune Cell Serum Replacement	2.5%
CTS OpTmizer T Cell Expansion Supplement	2.6%
CTS OpTmizer Pro Basal Medium*	Basal medium

* CTS OpTmizer Pro SFM is available with CTS OpTmizer Pro Basal Medium packaged in a 1 L BPC (Cat. No. A4966103) or a 1 L bottle (Cat. No. A4966101).

Cell expansion

Settings for the HyPerforma Glass Bioreactor (stirred tank)

Three days after seeding cells in the G-Rex vessels, the cells were inoculated into the 3 L glass bioreactor at a density of 2.5 x 10^5 cells/mL in 750 mL of manually prepared or perfusion-formulated expansion medium containing IL-2 at 10 ng/mL. The bioreactor settings are shown in Table 2, and the perfusion strategy for each bioreactor is summarized in Table 3.

The pump was set to add 60 mL of supplement per day if perfusion was performed at 100% of the vessel volume per day (VVD, 2.4 L) for a supplement concentration of 2.5%. Likewise, the pump was set to 30 mL per day if perfusion was performed at 50% VVD (1.2 L) to add the same supplement at a concentration of 2.5%. Dissolved oxygen (DO) was maintained at the indicated level with automated gas control, and the pH was kept at or above the indicated level through automated addition of 1 N NaOH. The agitation settings for the HyPerforma Glass Bioreactor are summarized in Table 4.

Settings for the rocking motion bioreactor

On day 3, cells were inoculated into 2 L perfusion bags at a density of 2.5 x 10^5 cells/mL in 1 L of manually prepared or perfusion-formulated expansion medium containing IL-2 at 10 ng/mL. Dissolved oxygen was maintained at the indicated level with automated gas control, and the pH was kept at or above the indicated level with automated addition of base. The agitation rate was set to 12 rpm at a 6° rocking angle. Perfusion was initiated when the cells reached a density of 2 x 10^6 cells/mL at the perfusion rates shown in Table 3.

Cell characterization

Cell growth and viability were monitored daily, and phenotype was assessed on day 10 using the Invitrogen™ Attune™ NxT Flow Cytometer. For phenotyping, cells were stained with Invitrogen™ Human CD3 Pacific Orange™, CD4 FITC, CD8 Pacific Blue™, CD62L APC, and CD27 PE.

Table 2. Settings for the HyPerforma Glass Bioreactor and rocking motion bioreactor.

Parameter	HyPerforma Glass Bioreactor	Rocking motion bioreactor
Inoculation	750 mL on day 3 post-activation (cell density: 2.5 x 10^5 cells/mL)	
pH	6.9–7.4	
Temperature	37°C	
Dissolved oxygen	30%	
O_2 delivery	Drilled-hole L sparger: 0–2,000 mL/min Headspace: 0–250 mL/min	Headspace (0–250 mL/min)
CO_2 (headspace)	6.6% of total gas	
Carrier gas	N_2 (headspace)	
Agitation	Dual pitched-blade impeller	Rocking motion
Volume adjustment	0.75 to 1.2 to 2.4 L*	0.3 to 0.5 to 1.0 L**
Working volume	2.4 L	1.0 L

* Bolus medium addition was performed on day 5 to 1.2 L and on day 7 to 2.4 L.
** Bolus medium addition was performed on day 5 to 0.5 L and on day 7 to 1.0 L.

Table 3. Perfusion parameters for the glass bioreactor and rocking motion bioreactor.

Glass bioreactor		Rocking motion bioreactor	
Vessel volume per day (VVD)	**Viable cell density (VCD)**	**Perfusion rate (L/day)**	**Viable cell density (VCD)**
50%	<2 x 10^6 cells/mL	0	<2 x 10^6 cells/mL
75%	6–12 x 10^6 cells/mL	0.5	2–5 x 10^6 cells/mL
100%	12–20 x 10^6 cells/mL	0.75	5–10 x 10^6 cells/mL
200%	>20 x 10^6 cells/mL	1	10–15 x 10^6 cells/mL
–	–	1.5	15–25 x 10^6 cells/mL
–	–	2	>25 x 10^6 cells/mL

Table 4. Agitation settings for the HyPerforma Glass Bioreactor. Tip speed was adjusted with bolus medium addition on days 5 and 7.

Volume	Agitation rate (120–435 rpm ramp)	Tip speed	Agitation rate (120–600 rpm ramp)	Tip speed
750 mL	120	0.35 m/s	120	0.35 m/s
1.2 L	278	0.82 m/s	278	0.82 m/s
2.4 L	435	1.27 m/s	600	1.76 m/s

Results

Cell growth and viability in a rocking motion bioreactor

Growth with perfusion in the Xuri Cell Expansion System W25 resulted in more than a 300-fold expansion after 10 days in culture (Figure 1A). Over 80% of the cells remained viable throughout the experimental period (Figure 1B). There was no significant difference in cell growth or viability for medium prepared in an open or closed configuration. The distribution of phenotypes was desirable in each case, with a high percentage of early memory cells and a low percentage of effector cells (Figure 1C).

Cell growth and viability in the perfusion-based 3 L HyPerforma Glass Bioreactor

Expansion in the glass bioreactor exceeded 600-fold after 10 days in culture, and over 80% of the cells remained viable over the experimental period (Figure 2A–B). The strategy for medium preparation appeared to have no significant impact on cell growth and viability. In each case, we observed a desirable distribution of phenotypes, with a high percentage of early memory cells and relatively few effector cells (Figure 2C).

Cell expansion was faster in the HyPerforma Glass Bioreactor than it was in the rocking motion bioreactor, but we are not attributing faster cell growth to this bioreactor. Cells expanded in the rocking motion bioreactor came from a different donor; therefore, there may have been significant differences in expansion capability due to donor-to-donor variability.

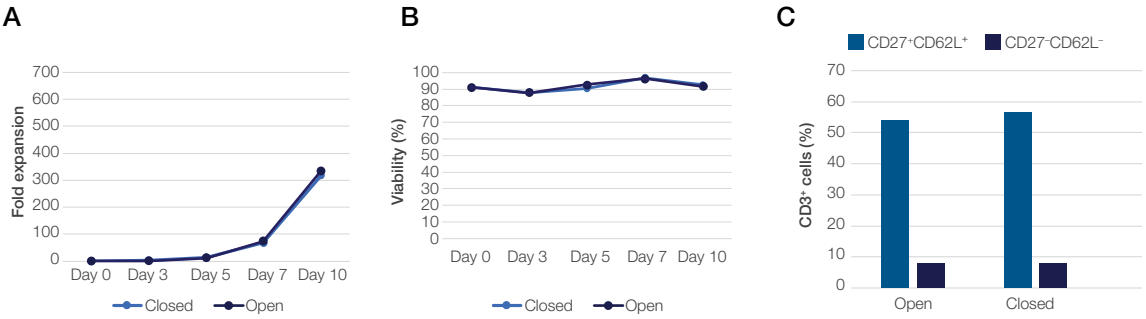

Figure 1. Cell expansion in the rocking motion bioreactor with medium formulated in an open or closed configuration. (A) Fold expansion and **(B)** viability of the cells were measured throughout the 10-day experiment. **(C)** Expansion yielded a desirable phenotypic distribution of a high percentage of $CD27^{+}CD62L^{+}$ early memory cells and relatively few $CD27^{-}CD62L^{-}$ effector cells.

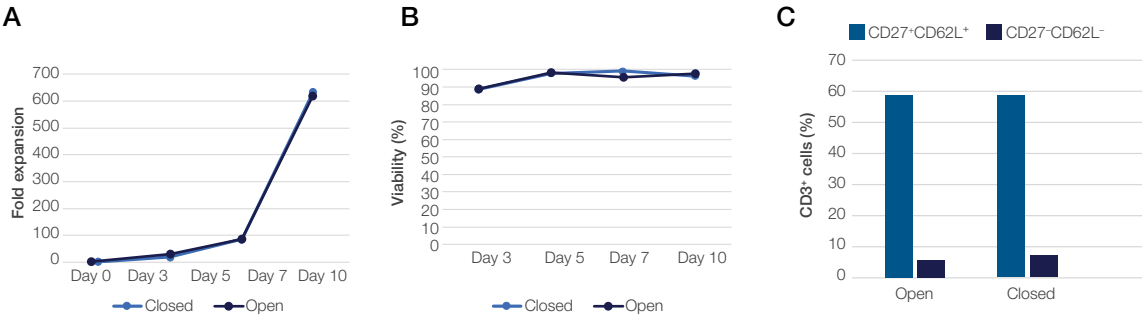

Figure 2. Cell expansion in the HyPerforma Glass Bioreactor with medium formulated in an open or closed configuration. (A) Fold expansion and **(B)** viability of the cells were measured throughout the 10-day experiment. **(C)** Expansion yielded a desirable phenotypic distribution of a high percentage of $CD27^{+}CD62L^{+}$ early memory cells and relatively few $CD27^{-}CD62L^{-}$ effector cells.

Discussion

Stirred-tank bioreactors like the HyPerforma Glass Bioreactor used in this study (Figure 3) have unique advantages for gas exchange when headspace is not sufficient to maintain the desired DO level or VCD. Stirred-tank bioreactors also enable more thorough liquid mixing with adjustable impeller and agitation settings.

For use with a fully automated and closed system, media and reagents must be available in BPC format along with tubing sets that are compatible with sterile welding. Many Gibco™ CTS™ media and reagents are available in BPC formats that are compatible with sterile welding and closed systems. All CTS reagents comply with industry standards and are backed by regulatory documentation in the form of drug master files, regulatory support files, certificates of analysis, and certificates of origin.

Our flexible BPC designs include 24 inches (61 cm) of C-Flex™ tubing to facilitate aseptic integration into CT workflows. We also offer a variety of connector options, including MPC quick connectors and female Luer-lock connectors (Figure 4).

With the growing availability of flexible, off-the-shelf options, CT developers have more ability than ever to streamline workflows and simplify their manufacturing processes. HyPerforma Glass Bioreactors are compatible with perfusion, which can eliminate the need for intermediate media preparation steps. Using them in combination with Gibco™ CTS™ basal media and supplements supplied in BPCs can help close this step in the CT manufacturing workflow. Removing open touchpoints can help improve CT safety and consistency, reduce costs, and reduce the risk of contamination.

Figure 3. HyPerforma Glass Bioreactor and G3Lab Controller with Thermo Scientific™ TruBio™ Bioprocess Control Software.

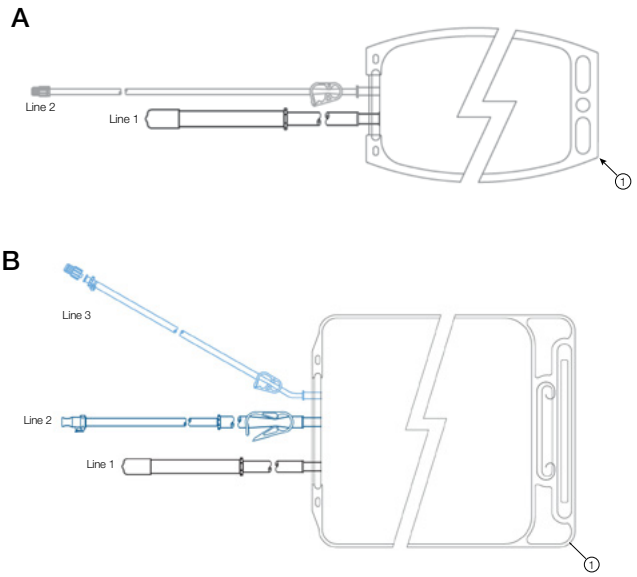

Figure 4. BPC options for closed-system media and reagents. **(A)** BPC for small-volume configurations. CTS OpTmizer Pro SFM (Cat. No. A4966103), Gibco™ CTS™ TrypLE™ Select Enzyme (Cat. No. A4738001), CTS GlutaMAX Supplement (Cat. No. A4737001), and CTS Immune Cell Serum Replacement (Cat. No. A4702901) are available in this format. **(B)** BPC for large-volume configurations. Only CTS Immune Cell Serum Replacement is available in this format.

Using a closed bioreactor with media components supplied in bags helps eliminate open touchpoints. This can reduce hands-on labor requirements and costs, improve consistency, reduce the risk of contamination, and ultimately help make CT drugs safer.

Closed systems

↑ Safety
Consistency

↓ Labor
Cost

Reference

1. Cell and gene therapy market size, growth, trends, forecast report 2022-2030. BioSpace, accessed January 19, 2023, **https://www.biospace.com/article/cell-and-gene-therapy-market-size-growth-trends-forecast-report-2022-2030**.

Ordering information

Product*	Quantity	Cat. No.
CTS DPBS, without calcium chloride, without magnesium chloride**	2 L	A1285602
CTS DPBS, calcium, magnesium, bag format**	2 L	A4737901
CTS AIM-V Medium, without phenol red, without antibiotics**	2 L	A4672701
CTS NK-Xpander Medium**	5 L	A5019002
CTS OpTmizer Pro SFM, bag format	1 L	A4966103
CTS TrypLE Select Enzyme	1 L	A4738001
CTS GlutaMAX Supplement	100 mL	A4737001
CTS Immune Cell Serum Replacement (SR)	250 mL	A4702901
	1 L	A4702902
CTS OpTmizer T Cell Expansion SFM, bag format	1 L	A1048503

* All closed-system CTS media and reagents are compatible with Thermo Scientific™ HyPerforma™ bioreactors.
** Products are compatible with the Gibco™ CTS™ Rotea™ Counterflow Centrifugation System and the Gibco™ CTS™ DynaCellect™ Magnetic Separation System.

Learn more at **thermofisher.com/closed-system-compatible-reagents**

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