Cell Incubation Outside of a Barrier Isolator using an Improved Conditioned Transport Chamber Expands Scale-Out Capacity for Massively Parallel Patient-Specific Batch Producton

Alicia D. Henn, Shannon Darou, Randy Yerden. BioSpherix, Ltd. Parish, NY

Abstract

Massively parallel patient-specific batch production of cellular therapeutics, such as in personalized CAR-T cell production for leukemia therapies, presents major technical challenges. Risks of contamination are reduced when a barrier isolator is used to surround the cellular production processes with a protective work tunnel. However, finite incubation space can limit scale-out of operations. In this study, we used an improved version of a Transportable Conditioned Cell Culture Chamber (TC4) to extend the protected environment of the barrier isolator to cultures transported to an outside incubator. We conducted cell growth kinetic studies using K562 cells, a cell line often used for optimizing T cell bioprocess operations. Cultures were incubated in three separate TC4 in external incubators, mimicking three separate patient-specific CAR-T cell cultures. Twice weekly over four weeks, cultures were moved into the isolator for systematic processing. The environment of each TC4 was conditioned by the barrier isolator before being returned to the exterior incubator. We found no statistical differences between the growth of K562 cells grown using the TC4 chambers and those incubated insde the barrier isolator full-time. Our disinfection protocol was effective at prevention of contamination of the barrier isolator by entering TC4 chambers. We conclude that the improved transportable chambers can extend the risk reduction of the barrier isolator to external incubators, providing vastly expandable parallel cellular therapy production capacity.

Background

Economically feasible cellular therapeutic cell cultures require efficient processing and expandable cell incubation capacity. The more parallel batches of cells in culture that can be sustained, the more autologous cellular products can be produced.[1] Barrier isolators reduce the risks of contamination for cellular products.[2] To overcome the inherent restrictions on incubation space in a barrier isolator, we designed transportable conditioned culture chambers that could safely house cultures outside of the barrier isolator while maintaining the atmosphere of the isolator.

Objectives

- **Assess K562 cell growth, comparing cultures housed in TC4 chambers in an external incubator with those grown in the barrier isolator.**
- **Monitor the Processing Chamber for contamination by entering TC4.**

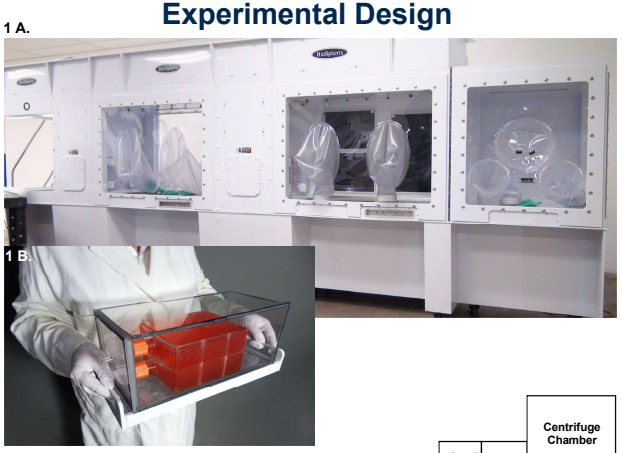
Experimental Design

K562 human leukemic cultures (ATCC Manassas, VA), in log-phase growth at 5% O_2, 5%CO_2 in RPMI-1640 medium (Invitrogen, Grand Island, NY) plus 10% FBS and glutamine (Sigma, St. Louis, MO), in a barrier isolator, were split into 6 T75 flasks (Celltreat, Shirley, MA). Three flasks were cultured in the isolator and three flasks were cultured in individual transportable conditioned cell culture chambers (TC4), mimicking individual patient cell cultures. The TC4 were housed in an external incubator.Twice a week, the TC4 were systematically moved from the external incubator, wiped in the Disinfection Chamber with SporKlenz (Steris, Mentor, OH), and the enclosed cultures subcultured in the Processing Chamber using good cell culture practices.The Disinfection and Processing Chambers were set for 5% O_2, 5% CO_2 and a constant 37ºC. The cells were counted with a hematcytometer and cell viability was assessed by trypan blue exclusion. Rodac contact plates and settling plates (BD Franklin Lakes, NJ) were used along with an air sampler (International pbi, Milan, Italy) for environmental monitoring of the Processing Chamber. Paired T-tests (Excel, Microsoft (Seattle, WA)) were used for comparisons.

Figure 1. Experimental Set-Up
(A) All cell handling was performed in a sealed barrier isolator that at constant 37ºC 20% O_2, 5%CO_2. (B) Cultures were transported to exterior incubators in TC4 that had been conditioned inside the isolator for consistent atmosphere.

Figure 2. High Throughput Cell Processing with Barrier Isolators and Transportable Conditioned Cell Culture Chambers. **(A, B) The first TC4 chamber (TC4-A) of the day was moved from the external incubator to the Laminar Flow Hood (LFH) and into Buffer Chamber 2. (C, D) After a 3-log buffer dilution to to replace air with filtered gas from tanks and reduce any airborne particles, TC4-A was moved into the Disinfection Chamber and all surfaces were wiped with SporKlenz-soaked gauze (shown here empty). (E-G) TC4-A was moved into Buffer Chamber 4 and a 3-log buffer dilution was performed. TC4-A was open to the Processing Chamber for access to cultures and equilibration with isolator atmosphere, but remained in the Buffer Chamber. Likewise, TC4-B was log-buffered in Buffer Chamber 3 and opened into Processing Chamber for cell processing. (H) TC4-A returned through BC 1 while TC4-C was log-buffered into the system.**

Experimental Design



Results

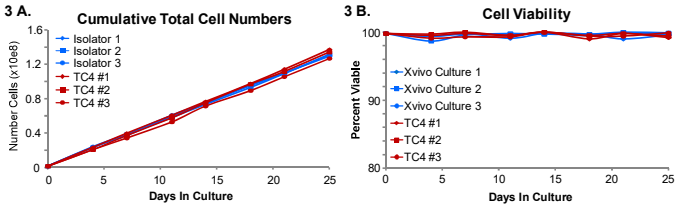


Figure 3. Equivalent Cell Growth
(A) K562 cells grown in the isolator (in triplicate) in 20% O_2 or in TC4 chambers conditioned in the isolator during routine cell culture manipulations and housed in external incubators had indistiguishable cumulative cell numbers (p>0.5, paired T-test, Day 25). Viability was greater than 98% at all times as measured by trypan blue exclusion (B). Cell cultures in separate TC4 chambers did not vary in cell growth characteristics increasingly over time (C), as would be expected if critical cell growth parameters varied between chambers. No microbial contamination of cell cultures was detected over 25 days of culture.

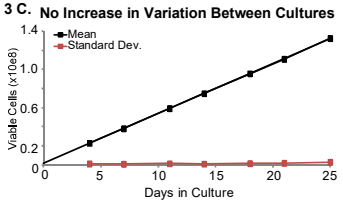


Table 1.

Day	4	7	11	14	18	21	25
Positive Control	95% confluent	95% confluent	95% confluent	95% confluent	95% confluent	95% confluent	95% confluent
Negative Control	<1 CFU	<1 CFU	<1 CFU	<1 CFU	<1 CFU	<1 CFU	<1 CFU
Left Floor	<1 CFU	<1 CFU	<1 CFU	<1 CFU	<1 CFU	<1 CFU	<1 CFU
Middle Floor	<1 CFU	<1 CFU	<1 CFU	<1 CFU	<1 CFU	<1 CFU	<1 CFU
Right Floor	<1 CFU	<1 CFU	<1 CFU	<1 CFU	<1 CFU	<1 CFU	<1 CFU
Air Sampler	<1 CFU	<1 CFU	<1 CFU	<1 CFU	<1 CFU	<1 CFU	<1 CFU

Table 1. Effective Disinfection of TC4 Chambers
An air sampler was set up in the Processing Chamber during all subculture session, pulling air onto a bacterial growth plate for monitoring viable airborne contaminants. No CFU were detected in the air at any time point in over three weeks of cell culture. Before end of session cleaning, Rodac contact plates were pressed to different areas of the chamber floor. The plates were sealed up in bags in the Processing Chamber, incubated for three days at 37ºC and then examined for colonies. The process was effective at preventing microbial contamination of the Processing Chamber by TC4 chambers.

Conclusions

- **K562 cell growth characteristics were equivalent in either TC4 chambers housed in an external incubator or the barrier isolator.**
- **The TC4 chamber disinfection process was effective.**
- **The system and process allows theoretically unlimited expansion of incubation capacity for massively parallel patient-specific batch production of cellular therapeutics while extending the reduced-risk isolator environment to the exterior incubator.**

References

1. Simaria, A.S., et al., Allogeneic cell therapy bioprocess economics and optimization: single-use cell expansion technologies. Biotechnol Bioeng, 2014. 111(1): p. 69-83.

2. US FDA, Guidance for Industry Sterile Drug Products Produced by Aseptic Processing - Current Good Manufacturing Practice. US Government Printing Office, 2004.