The Use of Conditioned Transport Chambers with a Barrier Isolator Provides Safe, Unlimited Expansion of Incubation Capacity

A. Henn, S. Darou, P. Easton, N. Ennist, R. Yerden. BioSpherix, Ltd.

Abstract

Critical to the success of both scale-up and scale-out of cellular therapy bioprocessing is incubator capacity. The ability to transport cell cultures safely between external cell culture incubators and a barrier isolator for cell manipulations allows unfettered expansion of cell incubation capacity. With the hypothesis that transportable conditioned culture chambers would maintain cell cultures with growth characteristics identical to cells maintained within the isolator, we tested three separate conditioned chambers. We found that K562 human leukemia cells maintained in an external incubator, but handled inside the isolator for all manipulations had growth characteristics indistinguishable from cells maintained within the isolator. A simple surface disinfection step prevented contamination of the cell processing chamber. We conclude that a transportable conditioned culture chamber provides easily expandable capacity to the isolator-based cell production system without compromising cellular growth. This system can be validated for clinical grade operations.

Background

For cellular therapies to be successfully translated into the clinic, safe and economically feasible cell culture processes must be developed. Larger batches of cells for allogeneic therapies allow for a smaller fraction of cells to be consumed for testing and decrease the cost of good sold. [1] Likewise, larger numbers of batches of cells in culture at the same time are needed for economical expansion of autologous cellular therapy cultures. [1] Whether allogeneic or autologous, products are exposed to fewer opportunities for microbial contamination by use of a barrier isolator.[2] However, incubation capacity is inherently limited within an isolator. Here, we designed a set of transportable conditioned culture chambers that could be used in conjunction with an isolator system for unlimited expansion of incubation capacity by safe transport of cell cultures to external incubators.

Methods

A single K562 human myeloid leukemia cells culture (ATCC, Manassas, VA) was split into 6 identical subcultures in T75 flasks (Celltreat, Shirley, MA). Cells were maintained in RPMI 1640 (Invitrogen, Grand Island, NY) medium supplemented with 10% FBS and L-glutamine (Sigma, St.Louis, MO) Three flasks were housed in three in separate transportable conditioned culture chambers, two were housed in the isolator incubator and one was maintained in an external incubator without the transport chamber. The cells were subcultured twice a week for four weeks. Simple manual cell counts using a Hemocytometer (VWR) with trypan blue exclusion for viability at each passage were used to generate doubling times and numbers of generations for each passage. Rodac contact plates and settling plates (Becton Dickinson, Franklin Lakes, NJ) were used along with an air sampler (International pbi, Milan, Italy) for environmental monitoring. ANOVA and one-way repeated measures ANOVA tests (Excel, Microsoft (Seattle, WA)) were used for statistical comparisons.

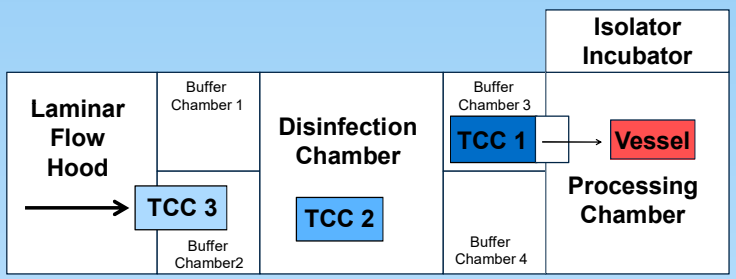

Figure 1a . Process Flow Through Isolator. After standard pre-procedure isolator cleaning, the first Transportable Conditioned Culture Chamber (TCC1) was brought from the external incubator through the Laminar Flow Hood (LFH). A three-log dilution buffer cycle in the Buffer Chamber replaced the chamber atmosphere with filtered air. After external surface disinfection, TCC1 was moved to buffer chamber 3 where a second three-log dilution was performed. TCC1 remained in this chamber throughout cell processing. During disinfection of TCC1, a second TCC was brought to the LFH from the external incubator and buffered into the system. While TCC2 was disinfected, a third TCC was placed in the Buffer Chamber so that at any one time, cultures in multiple transportable conditioned culture chambers could be systematically processed. Both the Disinfection Chamber and the Processing Chamber were maintained at 37ºC, 20% oxygen and 5% CO_2.

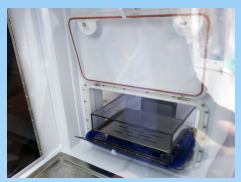

Figure 1b. Studies began with a large Transportable Conditioned Culture Chamber in use with a Larger Buffer Chamber. We began with contamination studies of the large TCC. We developed the half-size TCC for use within the half-size buffer chamber as part of this project. The data reported here were obtained using the half-sized buffer.

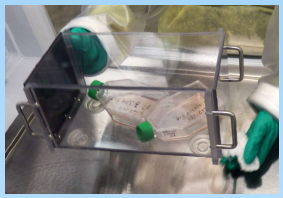

Figure 1c. Disinfection of Transfer Chamber External Surfaces. TCCs were surface disinfected by wiping with SporKlenz (Steris Life Sciences, Mentor, OH) soaked gauze. The chambers were allowed to dry before placement in the second set of buffer chambers.

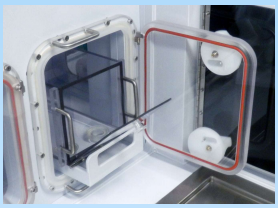

Figure 1d. The Transferable Conditioned Culture Chamber Atmosphere is Conditioned by Isolator During Cell Handling. After cell culture vessels were transferred from each TCC into the processing chamber, the TCC door was left open to condition the atmosphere inside. The cultures were replaced in the TCC after cell handling and the chamber was magnetically sealed for transport out of the isolator and back to the external incubator.

Results

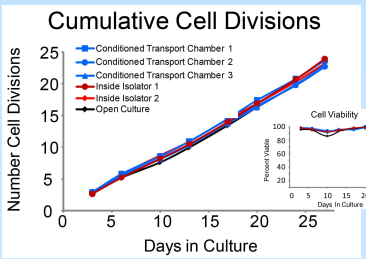

Figure 4. The Number of Cell Divisions in Three Separate CTCs was Comparable to Cells Grown in the Isolator and Cells Grown in Open Conditions. Using twice-weekly cell counts (Nx=cell number at timepoint, N0=starting cell number), we calculated the number of cell divisions at each timepoint. Cumulative cell divisions were quite similar between different growth chambers, isolator, and open (flask on exterior incubator shelf, subcultured in laminar flow hood) cultured cells. Cell viability dipped slightly when there was a change in FBS lots in use at Day7, but was greater than 90% in in all closed system cultures (inset).

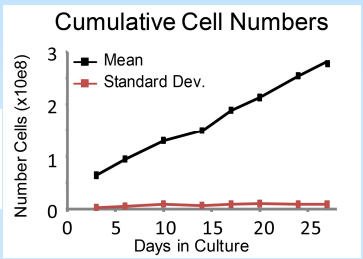

Figure 5. The Variation in the Average Cumulative Cell Numbers Did Not Increase Over Four Weeks. Even after 28 days in culture, the standard deviation of the cumulative cell numbers across all of the cultures did not increase. This suggests that there was no constant, continuing influence on cellular growth that would cause a divergence of population sizes in cultures maintained in different TCC, inside the isolator or in open culture.

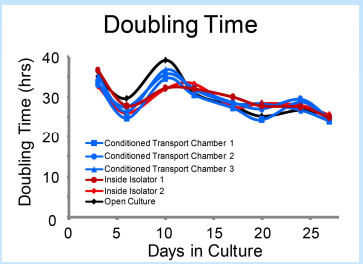

Figure 6. Cell Doubling Times Were Indistinguishable from Cells Maintained Within the Isolator. Doubling time was calculated using the above formula (N0 = starting cell numbers, Nx = cell numbers at each timepoint). There was no significant effect of culture conditions on cell doubling times [$F(5,42) = 0.1585$, $p > 0.05$] A one-way repeated measures ANOVA analysis also showed no statistical differences.

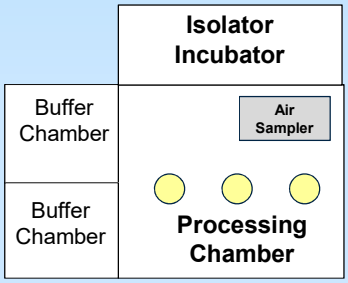

Figure 7. Environmental Monitoring of Bioburden Showed No Contamination of Processing Chamber Air or Floor. While no microbial contamination of cell cultures was evident over 4 weeks, we monitored air in the unit during each cell processing session. An air sampler was used to draw air onto a bacterial growth touchplate. In addition, after processing each TCC culture, touchplates were used to monitor three places on the chamber floor. Less than one CFU was found on each plate after incubation.

Conclusions

The use of Transportable Conditioned Culture Chambers results in cellular growth characteristics equivalent to cultures within a barrier isolator or in open culture conditions without contamination.

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