MASSIVELY-PARALLEL PATIENT-SPECIFIC CELLULAR THERAPEUTICS EFFICIENTLY SCALE IN CYTOCENTRIC ISOLATORS WITH A TRANSPORTABLE CONDITIONED CELL CULTURE CHAMBER

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Abstract

Massively-parallel patient-specific batch production of cells for CAR-T, and other gene and cellular therapeutics, centralized or distributed, will require replicable closed aseptic production lines. A modular barrier isolator can aseptically close any cell handling production process. Modular isolators scale efficiently by sequentially replicating each rate-limiting step within a process until all steps in production are at maximal capacity. The entire production line can then be cloned infinitely. Since incubation capacity is typically the first rate-limiting step within a process, one strategy for scaling up incubation capacity with isolators is aseptically closed culture vessel chambers that can be moved out to conventional incubators. The Transportable Conditioned Cell Culture Chamber (TC4) can aseptically enclose culture vessels inside the barrier isolator and maintain the aseptic environment as cells are transported to and from exterior incubators. We tested a TC4 capable of high gas exchange for assurance of equilibration and optimization in the largest of cell populations. To assess gas exchange, the TC4 chamber interior was equilibrated to 5% O2 inside the isolator before being closed for transport. After the filter covers were removed in the incubator to allow HEPA-filtered gas exchange, chamber oxygen levels equilibrated with the incubator within 30 minutes. To test cell growth, cultures of K562 cells were incubated in a TC4 or inside the barrier isolator at 5% CO2 19%O2, in triplicate. The cells in the TC4 were transported to the isolator for cell passaging. Environmental monitoring of the processing chamber was conducted at the end of each cell handling session. The resulting data show that cell growth over three weeks was statistically indistinguishable between the two conditions. Surface disinfection precented contamination of the barrier isolator by docking TC4 chambers. We conclude that the new TC4 chamber (patent pending) can keep cells in the external incubator aseptic while allowing full gas equilibration. This eases a typical operational bottleneck by allowing unlimited expansion of incubation capacity in the reduced-risk environment of a barrier isolator.

Introduction

Barrier Isolators, which physically separate cell cultures from personnel, are the best means to reduce contamination risks to patient cultures, and reduce cell handling risks to workers. They have been adopted for GMP-compliant clinical trials[1, 2]. and offer a modular design that can scale with operations as cell production processes move from the laboratory, through pre-clinical studies to clinical studies[3]. The Transportable Conditioned Cell Culture Chamber (TC4) was designed as a way to efficiently scale up the incubation step, combining the low-contamination-risk environment of the isolator with the economy of external room-air incubators. Each patient-specific cell culture enclosure can contain several cell culture vessels and be bar-coded for culture tracking. For cell cultures with high metabolic demands, we developed a TC4 with large square HEPA filters magnetically sealed to each end to allow for unlimited particle-free gas exchange between the incubator and the conditioned cell culture chamber. Magnetically sealed covers protect the HEPA filters during culture transport between the isolator and the incubator. Here we report on initial tests of the new device, assessing gas exchange with the incubator, cell growth, and the effectiveness of TC4 disinfection protocols.

Objectives

- **To measure the time to equilibrate the TC4 with the external incubator atmosphere**
- **To compare cell growth characteristics between cells grown with the TC4 and cells grown in the isolator full-time**
- **To assess the decontamination protocol**

References

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3.Yufit, T., P. Carson, and V. Falanga, Topical Delivery of Cultured Stem Cells to Human Non-Healing Wounds: GMP Facility Development in an Academic Setting and FDA Requirements for an IND and Human Testing. Current drug delivery, 2014. 11(5): p. 572-581.

Methods

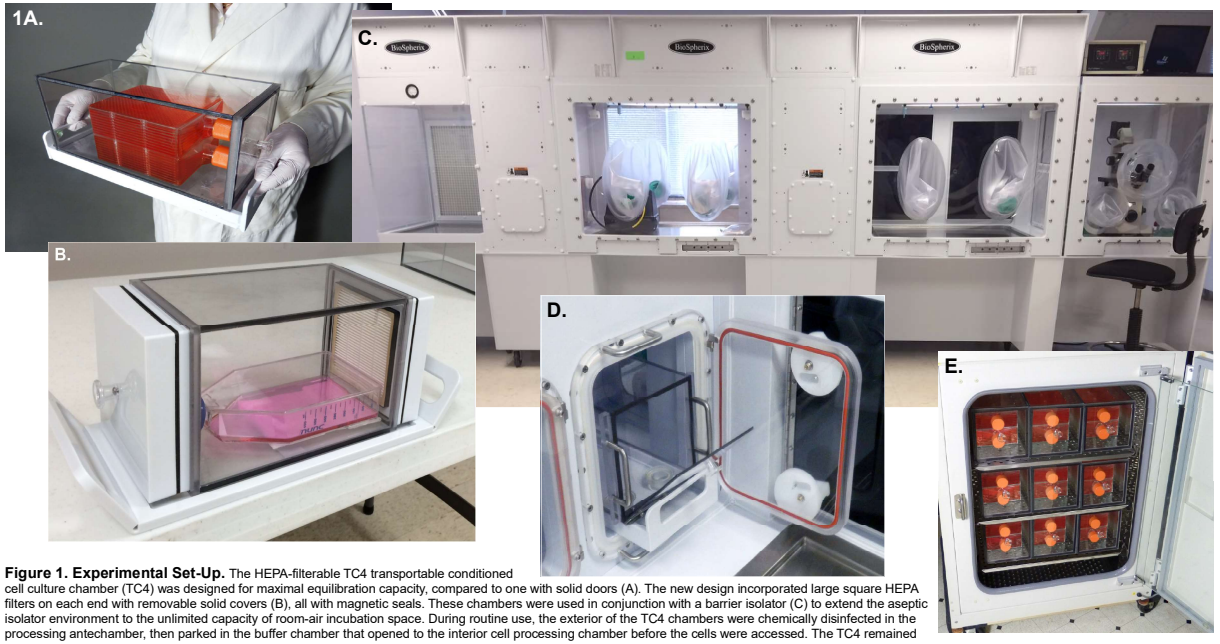

Figure 1. Experimental Set-Up. The HEPA-filterable TC4 transportable conditioned cell culture chamber (TC4) was designed for maximal equilibration capacity, compared to one with solid doors (A). The new design incorporated large square HEPA filters on each end with removable solid covers (B), all with magnetic seals. These chambers were used in conjunction with a barrier isolator (C) to extend the aseptic isolator environment to the unlimited capacity of room-air incubation space. During routine use, the exterior of the TC4 chambers were chemically disinfected in the processing antechamber, then parked in the buffer chamber that opened to the interior cell processing chamber before the cells were accessed. The TC4 remained open in the buffer chamber during cell handling for atmospheric conditioning by the inner processing chamber atmosphere of the isolator (D). After the cells were replaced inside the chamber, the TC4 was sealed again with fresh HEPA filters and returned to a conventional exterior incubator like the one pictured in (E). Contact plates and an air sampler were used to test for contamination of the processing chamber during cell handling.

Results

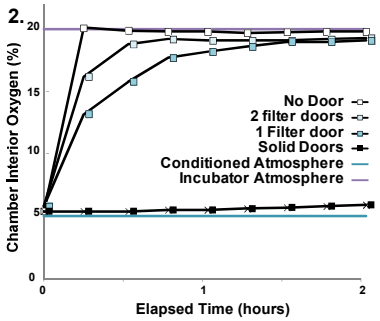

Figure 2. Equilibration with Incubator Air An oxygen probe was sealed into a test TC4 chamber through a small hole in the top surface. The chamber was equilibrated in the barrier isolator to 5% oxygen to assess equilibration with incubation air (~20% oxygen). The test chamber was assayed three times in each configuration; with no doors, with two covers in place over the filters, with one filter uncovered, and with two filters uncovered. Interior oxygen levels in the chamber were recorded every fifteen minutes. With filter doors uncovered on each end, the chamber equilibrated rapidly, reaching maximal O2 in about 30 min. The mean of the values for three trials is shown for each configuration.

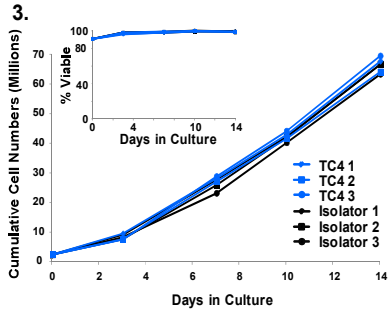

Figure 3. Equivalent Cell Growth in the Isolator or the TC4/Exterior Incubator. K562 human leukemia cells, used for process optimization in CAR T protocols, are an HSC-like hematopoietic tumor cell line. We seeded six T-75 flasks with 1x10e5/ml K562 and cultured three flasks in each condition; in the barrier isolator or in the TC4 chamber with filters. All cell handling was performed in the barrier isolator for routine cell counting and passaging twice weekly. Cell growth was statistically indistinguishable between the two groups (Day 14, paired T-test assuming unequal variances, (p=0.64)). Cell viability as assessed by trypan blue exclusion was greater than 90% for all cultures (inset).

Day	0	3	7	10	14
(+) Control	~90% confluent	~90% confluent	~90% confluent	~90% confluent	~90% confluent
(-) Control	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU
Left Floor	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU
Middle Floor	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU
Right Floor	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU
Gloves	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU
Air Sampler	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU

Table 1 Protocols Prevented Contamination of the Isolator by Entering TC4. The TC4 was brought into the HEPA-filtered laminar flow hood and decontaminated by wiping with SporKlenz-dampened gauze. The TC4 was moved into the airlock-like buffer chamber and a 3-log dilution of the air was performed with filtered, tanked medical gases to reduce any remaining airborne particles. In the processing antechamber, the TC4 was wiped again with SporKlenz-dampened gauze., then placed in the second buffer chamber where a second 3-log dilution was performed. Cell culture supplies were disinfected with the same method. During cell culture, an air sampler in the cell processing chamber continuously pulled air onto a bacterial culture plate. At the end of cell handling, one contact plate was pressed to each of three places on the processing chamber floor and to the isolator gloves. The contact plates were sealed and bagged before removal from the unit. Positive control plates were pressed to the open room floor. Plates were incubated for a minimum of four days before being visually inspected for colony growth. No colonies were detected on test or negative controls. No microbial contamination of any cell culture was evident at any timepoint. These findings support the ability of TC4 decontamination procedures to maintain the sterility of the isolator.

Conclusions

- **The filtered TC4 allows rapid equilibration of the chamber with HEPA-filtered incubator atmosphere**
- **Cells grow equally well in the isolator or in the exterior incubator using the filtered TC4**
- **Cleaning protocols were effective in preventing contamination of the barrier isolator and the cells**