Scaling of Massively Parallel Patient-Specific Cell Cultures with a Modified Transportable Conditioned Cell Culture Chamber

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Abstract

Barrier isolators, which separate the cell culture processing atmosphere from the bioburden of personnel, are the best means to reduce contamination risks. These isolators are currently being used for cGMP-compliant clinical trials[1,2]. Scaling cell production processes presents non-obvious restrictions. Compared to open processing, modular Cytocentric isoaltors can be replicated to scale proportionately with each stage in cell processing until all steps are accommodated maximally. This allows a process to efficiently and quickly scale with operations from the pre-clinical through clinical[3]. However, for processing of massively parallel patient-specific cell cultures, incubation capacity in the barrier isolator, unlike in an open room, can be a major bottleneck. Inexpensive and infintely elastic incubation capacity can be provided by existing external incubators if cultures can be safely transported to and from the isolator for processing. We tested a modified transportable conditioned cell culture chamber (TC4) designed to enclose cell cultures inside the exterior incubator and fit through the airlocks of a barrier isolator to safely deliver cells to the interior for processing. We have previously published on good cell growth using this processing system to expand K562 cells, a hematopoietic stem cell-like cell line that has been used as a surrogate for CAR-T cell processing. In this study, we addressed sterility concerns by running mock production runs with a highly permissive color-changing bacterial broth. We ran three production runs, moving mock cultures between the barrier isolator and the external incubator with the TC4 transport chamber. We took samples fo the final mock cell product, sealed them into sterile vials, and incubated them long-term monitoring for bacterial growth. We also performed environmental monitoring of the barrier isolator processing chamber with an air sampler and contact plates. Positive controls were all yellow and turbid. Negative samples and all test materials were negative for microbial growth. We concluded that this transport chamber could help safely alleviate the bottleneck in cell production presented by the unique needs of massively-parallel patient-specific cell incubation.

Background

- Scale-Up of massively-parallel patient-specific cell therapeutic production proceeds smoothly until a rate-limiting step is encountered as a challenge to efficiency and profitability
- Modular barrier isolators provide easy scale-up of all cell handling steps while maintaining full-time aseptic conditions
- Incubation capacity can be the rate-limiting step for scale-up of cellular therapeutic cell processes in modular isolators
- Flexible incubation capacity can be provided by exterior incubators
- We performed this study to evaluate the ability of Transportable Conditioned Cell Culture Chambers (TC4) to allow clean transport of cell cultures to an exterior incubator to combine the best of the barrier isolator with the flexible capacity of external incubators

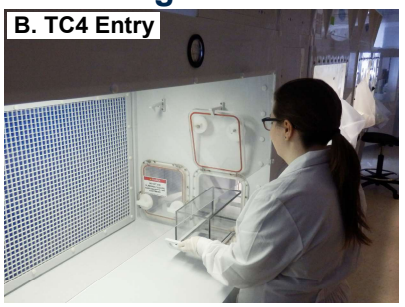
Objectives

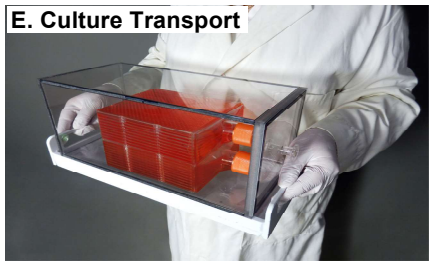
- Perform 3 mock cell production runs using the Transportable Conditioned Cell Culture Chamber (TC4) and the XVIVO System® barrier isolator, using highly permissive TSB broth (media test)
- Assess contamination of mock final product samples
- Monitor the Cell Processing Chamber of the XVIVO System for contamination by TC4

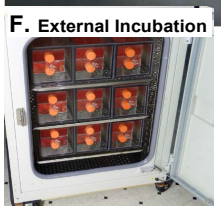
References

1. Mei, S.H., et al., Isolation and large-scale expansion of bone marrow-derived mesenchymal stem cells with serum-free media under GMP-compliance. mortality, 2014. 40: p. 1.
2. Marathe, C.S., et al., Islet cell transplantation in Australia: screening, remote transplantation, and incretin hormone secretion in insulin independent patients. Horm Metab Res, 2015. 47(1): p. 16-23.
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.

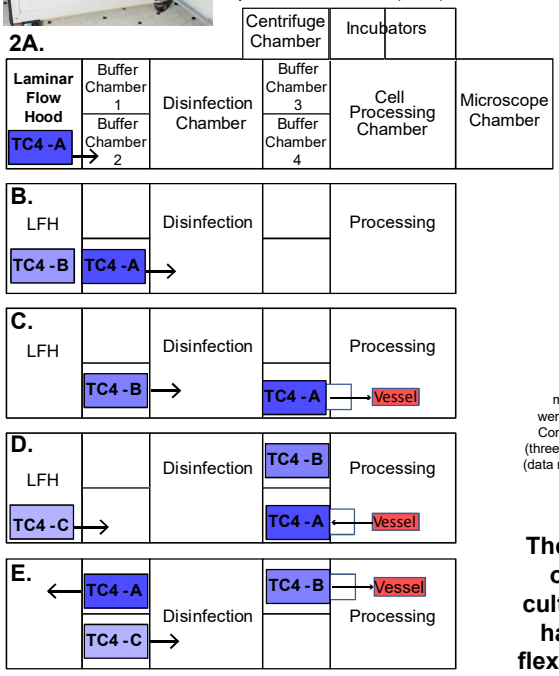
Experimental Design - Cell Processing







Mock cell production runs were conducted using highly permissive color-changingTSB bacterial broth (bioMerieux, France). Mock non-adherent patient cultures were subjected to a routine cell expansion protocol with cell passaging procedures on two separate days and cell harvest on a third day, with all cell handling performed in the in the Xvivo barrier isolator (1A). Cultures were transported to an exterior incubator in individual TC4 chambers. The TC4 were systematically moved from the external incubator, loaded into the Xvivo (B), wiped in the Disinfection Chamber with SporKlenz (Steris, Mentor, OH) (C), placed in buffer chamber 4 and opened for equilibration with the processing chamber atmosphere (D). The enclosed mock cultures were handled in the Processing Chamber using good cell culture practices, and transported (E) out of the Xvivo to exterior incubators for housing (F) . (2A-E) Individual batches in enclosing TC4 were moved through the Xvivo serially for efficient processing. The chambers were set for a constant 20% O_2, 5% CO_2, 37ºC. The harvested mock cultures (TSB) were assessed for microbial contamination at day 7 and 20. Rodac contact plates (BD Franklin Lakes, NJ) with an air sampler (International pbi, Milan) were used for environmental monitoring.



Results

All positive control vials in all three batches were turbid and yellow, positive for microbial growth (far right). All test samples of mock cell cultures in all three batches were clear and pink, negative for microbial growth, at 7 and 20 days of sample incubation. Contact plates used to assay for microbial contamination of the Processing Chamber floor (three places), gloves, and the atmosphere (air sampler) were all negative for microbial growth (data not shown). Postiive control plates, touched to surfaces outside of the Xvivo had colonies.

Conclusions

The TC4 Transport Chamber maintained sterility of cultures, reducing contamination risks to cultures, while safely connecting the aseptic cell handling space of the barrier isolator with the flexible incubation capacity of external incubators