Cell Culture Contamination Risk Reduction for Human MSC, Progenitor Cell, and CAR-T Production by Full-Time Separation of the Room Air and Cell Processing Environments

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Abstract

For clinical translation of cellular therapeutics, the risk of microbial contamination of the cell product must be addressed. Patient cell cultures are most exposed to risks during open cell handling steps of cell isolation, expansion, gene modification, differentiation, selection, or harvest. Even when performing manipulations in a cleanroom, personnel present the largest contaminant source. A barrier isolator physically separates the cell environment from personnel-borne bioburden. We set out to determine if performing routine cell production in a Cytocentric barrier isolator could control the risk of contamination. We performed three different routine cell production protocols using a permissive microbial culture broth as a cell culture surrogate. The first protocol was a cell thaw. The second was a multi-step production of an adherent cell type like mesenchymal stromal/stem cells (MSC). The third protocol was passaging of a non-adherent cell type like CAR-T cultures. Samples of final preparations were sealed into sterile vials for long-term incubation. Each protocol was performed at least three times. Contact plates were used to assess the sterility of processing surfaces inside the isolator during and after the process was completed. Positive controls were injected with 0.1ml of tap water or exposed to the room outside of the isolator. After incubation for at least five days, test broth samples and contact test plates showed no contamination (0 CFU) while positive control samples and plates showed vigorous microbial growth. After incubation, test broth samples were negative for microbial growth. Full-time particle sensors on the isolator modules also showed that non-viable particles generated during open handling steps were filtered rapidly from the air by CRAC air filtration system. We concluded that separation of the cell processing of the cell product surrogate from the room air environment effectively prevented microbial contamination.

Background

- Translation of cell therapies from the lab to the clinic requires translation of pre-clinical processes to GLP/GMP standards
- The Cytocentric barrier isolator continually HEPA-filters the cell processing air to ISO 5 levels
- Personnel are excluded from the isolator environment, so the next biggest source of bioburden are surfaces of entering materials. Effective cleaning of surfaces are necessary to reduce the risk of contamination

Objectives

- Perform three trials each of three different mock cell handling protocols in the Cytocentric barrier isolator with TSB broth to assess for microbial contamination
- Test microbial contamination of barrier isolator with contact plates and air sampler and analyze particle monitor data during cell handling

Experimental Design

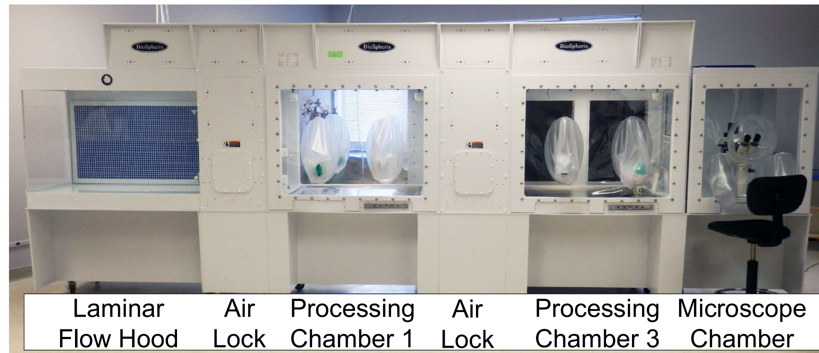
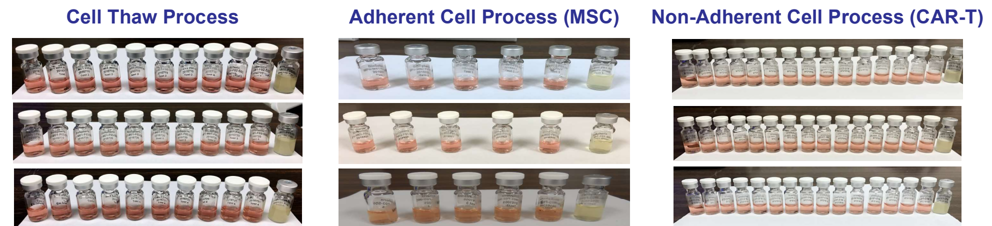


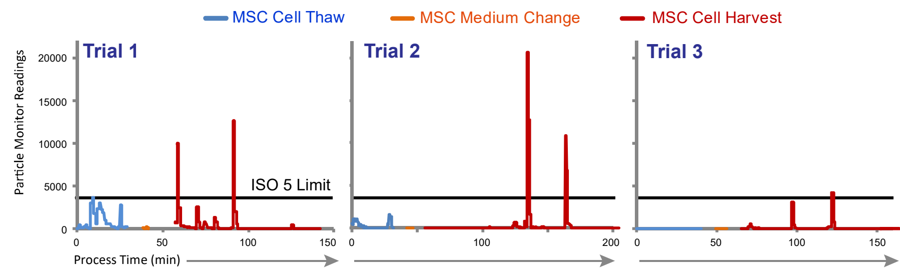
Figure 1. Experimental Design. Three different mock routine cell processes using permissive TSB broth (Biomerieux-USA) were performed in a Cytocentric isolator. The internal atmosphere was provided by medical-grade, tanked gases and was continuously HEPA-filtered. All materials were surface decontaminated by wiping with SporKlenz (Steris, Mentor, OH)-soaked gauze in the laminar flow hood unpackaged in Process Chamber 1 (PC1). Open handling steps were performed in PC3. Mock cultures were incubated in the incubators (black doors) in PC3. Samples were centrifuged in a chamber behind the unit. Final mock cell preparations were sealed into vials and incubated at least 20d.

Results

2A. Product Media Fill Test



B. Processing Chamber 3 Air Particle Monitoring - Adherent Cell Process **(MSC)**



C. Surface Monitoring

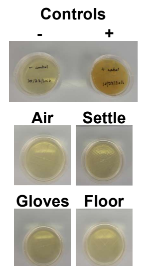


Figure 2. (A) Positive control vials were turbid yellow, positive for microbial growth (far right). All negative and test samples of mock cell preparations were clear pink, negative for microbial growth, at 7 (not shown) and 20 days (shown) of sample incubation. (B) Full-time particle monitoring showed that when non-viable particles were generated inside the isolator by the opening of packaging or other manipulations, the particles were rapidly cleared from the processing chamber atmosphere. (C) Contact plates used to assay for microbial contamination of the Processing Chamber floor (three places), gloves, and the internal atmosphere (air sampler) were all negative for microbial growth. Postiive control plates, touched to room air surfaces outside of the Xvivo were positive for microbial growth (One set shown).

Conclusion

The use of the Cytocentric barrier isolator and its protocols for surface disinfection prevented microbial contamination of the product and the processing chamber.

References

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