LAYERED PROTECTIONS FOR ASEPTIC ADIPOSE-DERIVED REGENERATIVE CELL PROCESSING

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Abstract

Introducing automation into a clean environment for cGMP-compliant cell processing is of clear benefit for more reproducible, reliable, and cost-effective cell products. Enclosure of functionally closed systems in the Xvivo System may better satisfy GMP regulatory requirements of some countries by adding an additional aseptic barrier. The Xvivo System completely controls the cell environment, including temp, particles, and the gas atmosphere. A continuously recirculating air cleaning (CRAC) system employs constant HEPA filtration to protect the cell environment. Here we tested conditions during operation of the Cytori Celution inside an Xvivo System for cGMP cell processing. The Celution prepares adipose-derived regenerative cells (ADRCs) from adult tissue for autologous cell therapies, reducing risk of graft rejection or disease transmission. Automated cell processing included mechanical rocking of an enzymatic digestion chamber, actuation of valves and pumps for fluid transfer, and repeated rounds of centrifugation. During all cell processing activities, the Xvivo System was able to maintain particles below the ISO5 limit of 3500 particles/m^3 and maintain heat wihin optimal operating ranges. Touchplate environmental sampling showed that the chamber interior was not contaminated during operation. We concluded that the combination of these two technologies maintains an aseptic environment around automation for optimal protection of cells during cGMP cell processing.

Background

- **Automation can improve reproducibility in cell handling[1]**
- **Equipment with moving parts generate particles[2]**
- **Not all particles constitute contamination events for cell processing, however being able to monitor particles adds assurances and records of ISO 5 conditions**
- **Functionally closed cell processing eliminates contact of the cell product with the environment except during direct access of the cell product.[3]**
- **Enclosing a functionally closed system with a completely closed environment for the entire production process makes a fully closed and monitored system for better protection of patient tissues and cells**
- **A small modular barrier isolator can be used at the point of care or combined with a larger clinical laboratory for culturing cells *ex vivo* in an enclosed, monitored system**

Objectives

- Operate the Cytori Celution enclosed in the Xvivo System
- Assess the internal chamber environment for non-viable particles, heat and contamination

References

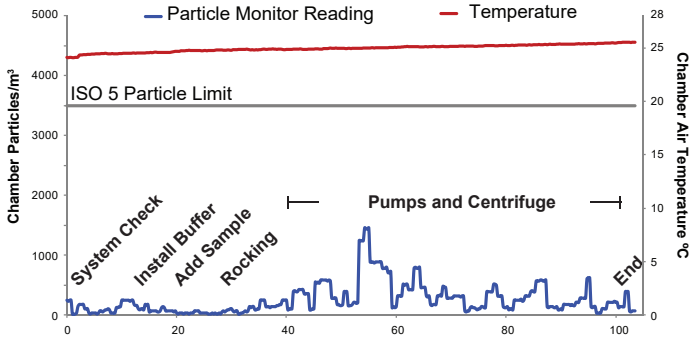
1. Ball O, Robinson S, Bure K, Brindley DA, Mccall D: Bioprocessing automation in cell therapy manufacturing: Outcomes of special interest group automation workshop. Cytotherapy 2018, 20(4):592-599.
2. Reda A, Bowen R, Westcott V: Characteristics of particles generated at the interface between sliding steel surfaces. Wear 1975, 34(3):261-273.
3. Hourd P, Chandra A, Medcalf N, Williams DJ: Regulatory challenges for the manufacture and scale-out of autologous cell therapies. 2014.

Experimental Design

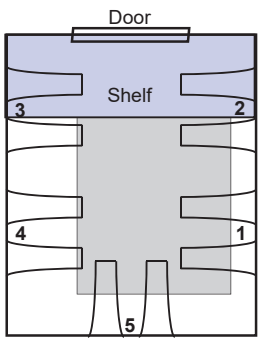
Figure 1. Experimental Design. The Cytori Celution 800/CRS was installed in an Xvivo System barrier isolator to surround the functionally closed system in an added layer of environmental protection and provide a fully closed and fully monitored solution for cell isolation. The air inside the chamber was from tanked, medical grade gases and was HEPA filtered with the Continuously Recirculating Air Cleaning (CRAC) system. The interior of the chamber including shelves, gloves, and sleeves were wiped with gauze dampened with disinfectant (SporKlenz, Steris). All test materials and packaging on disposable single-use consumable materials were also surface disinfected before import including bags of Ringers Lactate buffer pre-warmed to 37 ºC. Commerically-prepared rice pudding was loaded into 50ml syringes outside of the system and used as a test lipoaspirate substitute. The Celution's fully automated cell processing was completed in less than 90 min. Standard Rodac touchplates were used to sample surfaces inside the Xvivo System for microbial contamination.

Results

2A



B



C

	CRAC Off	CRAC On
Glove Set #1	< 1 CFU	< 1 CFU
Glove Set #2	< 1 CFU	<1 CFU
Glove Set #3	1 CFU	< 1 CFU
Glove Set #4	< 1 CFU	< 1 CFU
Glove Set #5	<1 CFU	< 1 CFU
Shelf	<1 CFU	< 1 CFU
Pos Control	~95% confl	~95% confl
Neg Control	<1 CFU	< 1 CFU

Figure 2. Optimal conditions for cell processing with layered protections. (A) During fully automated cell isolation any nonviable particles generated by motors were swept from the internal chamber atmosphere by the CRAC HEPA filtration system, maintaining an ISO 5 environment around the functionally-closed fully automated process. Chamber temperatures were maintained within optimal operating range. (B-C) Surfaces for environmental monitoring included the glove fingertips and the shelf inside the isolator. Touchplates were incubated for a minimum of 5 days before scoring. Positive control touchplates were applied to surfaces outside of the barrier isolator. Negative control plates were not exposed. One CFU was detected on one gloveset before the CRAC system was turned on, otherwise samples were negative.

Conclusion

The layered protections created by placing a functionally closed automated cell processing system within a small barrier isolator provides a completely closed cell processing environment wherever it is needed