Cytocentric Isolator Increases Cellular Yields Compared to Open Processing and Increases Biosafety for Handling Genetically Engineered Cells

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Abstract

Cell culture processing in a room-air biologic safety cabinet (BSC), even in a clean room, creates multiple risks for both cell cultures and users. Humans can be exposed to genetically-modified cells, viruses, and other biological hazards during suboptimal (open) cell handling in a room air BSC. The cell cultures can also experience sub-optimal room air during cell handling in the BSC which creates a contamination hazard. In addition, even in the cleanest clean room, cells are exposed to room air temperatures and gas levels. Until cells re-equilibrate with the incubator after handling, cells are chilled, hyperoxic, and hypocapnic compared to physiologic conditions. A barrier isolator (closed cell processing) creates a constant physical barrier between cells and users. This reduces the risk of air mixing between cells and the room without any dependence upon laminar air flow in a BSC or a costly whole-room HVAC system. We undertook the following study to test the hypothesis that use of a barrier isolator, during cell handling as well as incubation, could improve cell growth by providing unbroken physiologic conditions to cells. We divided a single K562 leukemic cell culture into two sets of triplicate cultures in T-75 flasks, three for growth in a barrier isolator and three for growth in a standard room-air incubator equipped with an inner chamber (C-Chamber) for control of gases. Both sets of cultures were incubatedat 5% CO2, 5% O2, 37°C and tracked for cell growth and viability over two weeks. For routine subculturing twice weekly, the cultures in the isolator were handled in the processing chamber of the isolator in conditions identical to incubation conditions. The cultures in the room-air incubator were handled in a standard room-air BSC (open). While cell viability was high in both sets of cultures, we found that cell handling under continuous physiologic conditions produced statistically higher cell yields over time (p=00086, Day 14, two-tailed paired T-test unequal variances). No contamination events occurred in either set of cell cultures. Particle count data indicated no breach of functional separation between room air and cell culture atmosphere. We concluded that cell handling in unbroken physiologically relevant conditions produced better cell growth while reducing risks to cells and cell culturists. We predict that as cells become increasingly valuable, a total quality approach will result in not only better cell growth, but also lower biosafety risks.

Background

Laboratory aerosols are ubiquitous, undetected, and the probable cause of LAI in workers whose only risk factor is working with an agent [1]. Workers, particularly those that are not vaccinated against potentially infectious laboratory materials, are at a high risk of infection[2]. Now with new vectors available and powerful new genetic modification techniques like CRISPR, there are new, unknown risks added onto the risks of handling patient-derived cells, such as infection with Hepatitis or HIV.

Barrier isolators that provide a physical separation of room air and cell processing atmosphere inherently enhance laboratory biosafety. Full-time control of the cell processing atmosphere allows for full-time optimal conditions for not only incubation, but any kind of cell handling machines like cell sorters, washers or separators that are suitable for clinical grade cell production. The modular nature fits any cell production process. Critical cell parameters temperature, CO_2 and O_2 are suboptimal during conventional handling and machine processing in room air, with optimal conditions only part-time during incubation. We hypothesized that full-time control of conditions for cells grown at physiologically-relevant oxygen levels would also result in better cell growth than part-time control.

Objectives

To assess the effectiveness of the physical separation between the cell handling environment and the room air

To assess K562 cell growth over time, comparing cultures incubated and handled in the full-time control of the barrier isolator with those incubated at 5% O2 in the incubator and handled in HEPA-filtered room air conditions (BSC)

Experimental Design

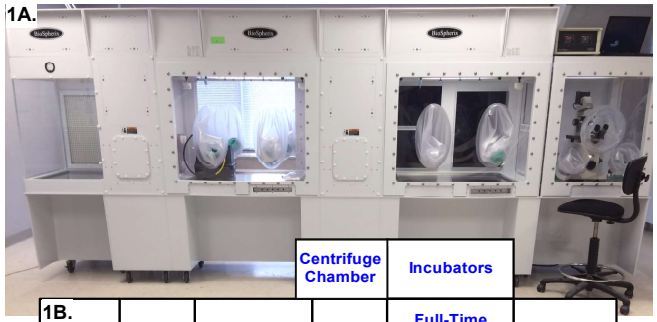

Table 1	Part-Time Physiologic Simulation			Full-Time Physiologic Simulation		
	Temp (°C)	CO2 (%)	Oxygen (%)	Temp (°C)	CO2 (%)	Oxygen (%)
Incubation	37	5	5	37	5	5
Handling	20-25	<0.5	~20	37	5	5

Figure 1. Experimental Set-Up. K562 cultures were split into two conditions; in part-time control of conditions in standard laboratory with an oxygen-controlled incubator subchamber, or full-time control in the barrier isolator (1A), a cell culture facility separated from room and infused with triple-filtered medical-grade tanked gases (1B). Each module in the isolator is separately controlled for temperature, particles, and atmosphere (1C). The entrance laminar flow hood (LFH) was used to manually surface decontaminate materials entering the unit with cotton gauze dampened with SporKlenz (Steris, Mentor, OH). The buffer chambers (airlocks) replaced air from the LFH with filtered, tanked gases.The first processing chamber was used as an additional disinfection chamber for a second manual disinfection step before materials were introduced into the cell processing chamber via a second set of buffer chambers. For full-time control, all cell handling was conducted at optimal conditions (Table 1).

References

1 CDC. Biosafety in Microbiological and Biomedical Laboratories. HHS Publications No. (CDC) 21-112 5th Edition (2009).
2 Rusnak, J. M. et al. Risk of occupationally acquired illnesses from biological threat agents in unvaccinated laboratory workers. Biosecur Bioterror 2, 281-293, doi:10.1089/bsp.2004.2.281 (2004).

Results

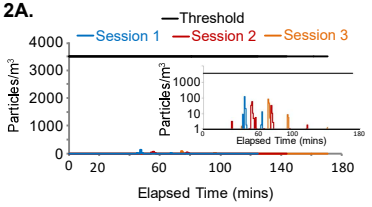

Table 2 Session	1	2	3
Pos Control (confluence)	80%	95%	85%
Neg Control (CFU)	<1	<1	<1
PC3 - Left Floor (CFU)	<1	<1	<1
PC3 - Middle Floor (CFU)	<1	<1	<1
PC3 - Right Floor (CFU)	<1	<1	<1
PC3 - Gloves (CFU)	<1	<1	<1
PC3 - Air Sampler (CFU)	<1	<1	<1

Figure 2. Cell Processing Atmosphere is Effectively Separated From Room Air. Full-time particle detectors in the barrier isolator did not detect particles leaking from room air into cell processing chamber (2A). Small numbers of non-viable particles were generated by motion of the integrated sleeves and gloves during normal cell handling (log-scale inset) (2B). Environmental monitoring using touchplates and an air sampler that drew air over a touchplate during cell handling detected no viable CFUs in the cell processing chamber (Table 2) over three sessions.

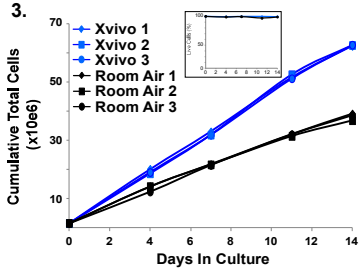

Figure 3. Increased Cell Yields in Full-Time Optimal Conditions without Obvious Cytotoxicity. K562 human leukemia cells were incubated in T-75 flasks at 5% CO_2 5%O_2 in RPMI-1640/10% FBS/L-glutamine (Gibco/Thermo). Cultures were split into triplicate flasks in either part-time optimal conditions with handling in a room air BSC or in full-time optimal conditions for both incubation and cell handling in the barrier isolator. Cells were counted at twice-weekly cell passaging sessions using a microscope and trypan blue (Krackeler, Albany, NY). Yields were significantly higher in full-time optimal conditions than in part-time optimal conditions (p=00086, Day 14, two-tailed paired T-test unequal variances). There was no obvious cytotoxicity (inset).

Conclusions

The cytocentric isolator provided effective separation of room air and cell processing atmosphere, enhancing biosafety for personnel handling genetically engineering cells

Nonviable particles generated by glove motion were rapidly eliminated

Full-time physiologic simulation with unbroken control of critical parameters during cell handling produced increased cell yields over part-time physiologic simulation

This was a cytostatic effect, not obvious by cell viability at any one time