Neutralizing the Negative Effects on Cells from Airborne Particles Generated by Cell or Tissue-Based Screening Automation

Alicia D. Henn[1], Paula Easton[1], Richard Ruffos[1], Brian Handerhan[2], George Muschler[3], Randy Yerden[1]

[1]BioSpherix, Ltd. Parish, NY [2]Parker Hannifin, Cleveland, OH [3]Cleveland Clinic, Cleveland, OH

Abstract

Environmental enclosures that control O_2 and CO_2 levels are essential for maintaining cells in a physiological milieu. O_2 levels *in vivo* are far lower than room air. The exposure of cells to supraphysiologic room air sharply increases intracellular reactive oxygen species[1], stressing the cells and skewing cell-based toxin-screening results[2]. Similarly, most cell culture media rise in pH when exposed to room air, unless CO_2 level is controlled, further stressing cells. Environmental enclosures also offer the opportunity to better maintain an aseptic environment, reducing the risks of contamination of both cells and equipment. Automation involving repetitive, high frequency, high speed robotic movement can also threaten the biological environment. Any automation equipment will generate some airborne particles. As a result, environmental particle generation and control becomes important to measure and manage. We enclosed the Cell X automated colony picking system in an Xvivo Cytocentric System with full-time O2, CO2, pressure, and relative humidity controls. To manage the risk of particles, we added a Vertical Laminar Flow (VLF) system, which pulled the chamber atmosphere vertically downward around the Cell X device through a vented floor, returning air through HEPA filters for full-time particle control. Our hypothesis was twofold: 1) particles associated with the start-up and operation of the Cell X could be measured, and 2) using the VLF system, an atmosphere of particles 0.5 microns or larger would be maintained below ISO 5 levels of 3520 particles/m^3. We positioned particle sensors on the front and rear of the Cell X inside the Xvivo chamber, and ran the Cell X device under high stress conditions, simulating a near maximal intensity of operation. We found that measurable particle generation associated with the Cell X initiation and operation. However, with the VLF in operation, the concentration of particles was controlled well below the ISO 5 limit. These data demonstrate the ability to control automation-generated particles inside the barrier isolator, even under high intensity movement conditions.

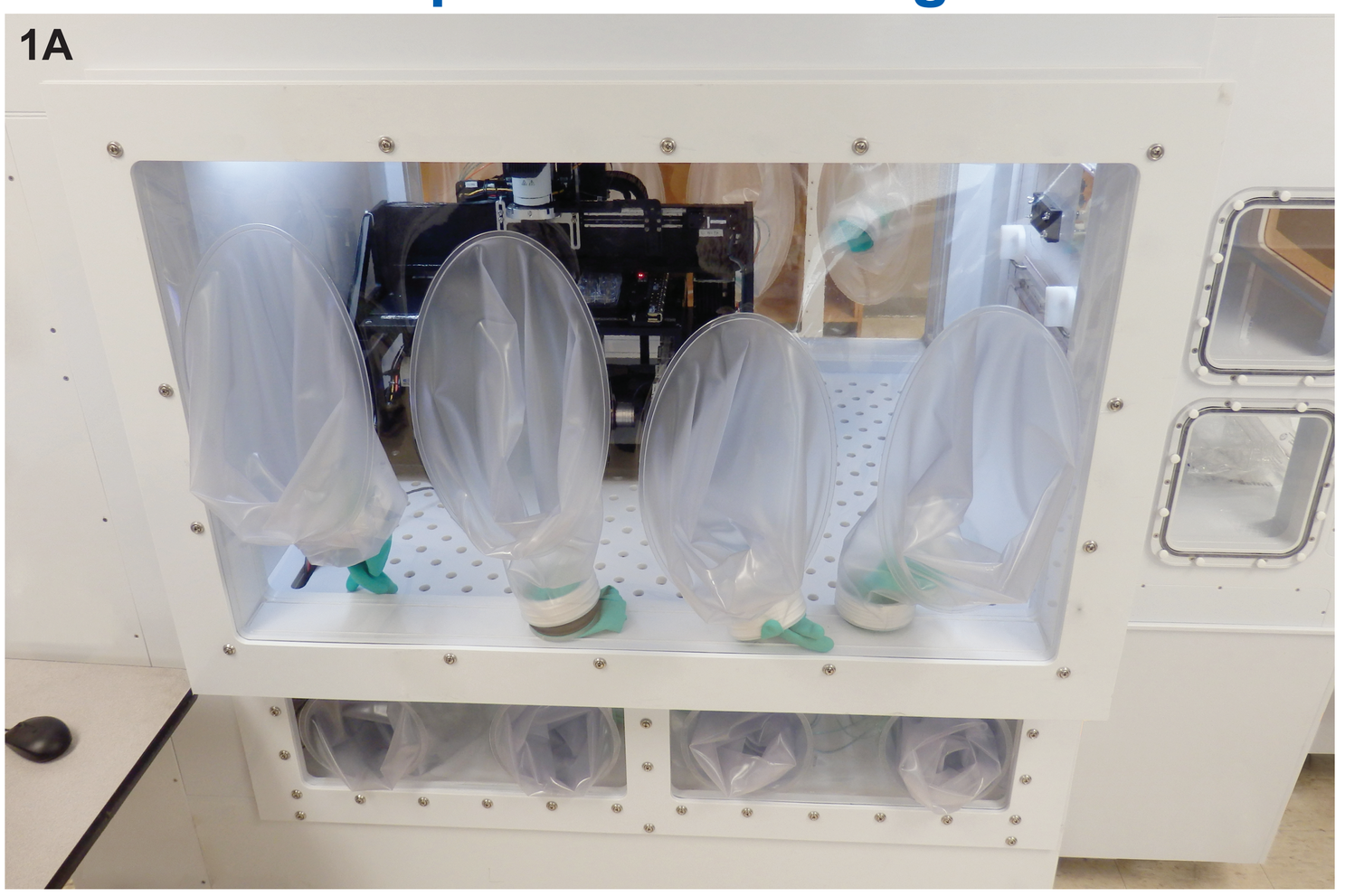
Background

- Room air oxygen is much higher than the physiologic environment, which can raise intracellular reactive oxygen species to detrimental levels[1]
- Supraphysioxic room air can skew cell or tissue-based screening assays[2]
- Enclosing automated systems can provide a separation from room air and allow control of physiologic O_2 and $CO_{2,}$. However, this can also concentrate automation-generated particles, creating a hazard for cells
- We added a Vertical Laminar Flow (VLF) System to the Xvivo for particle control

Objectives

- Measure particle generation by the Cell X
- Assess particle control by the Xvivo with or without the VLF System in operation

Experimental Design



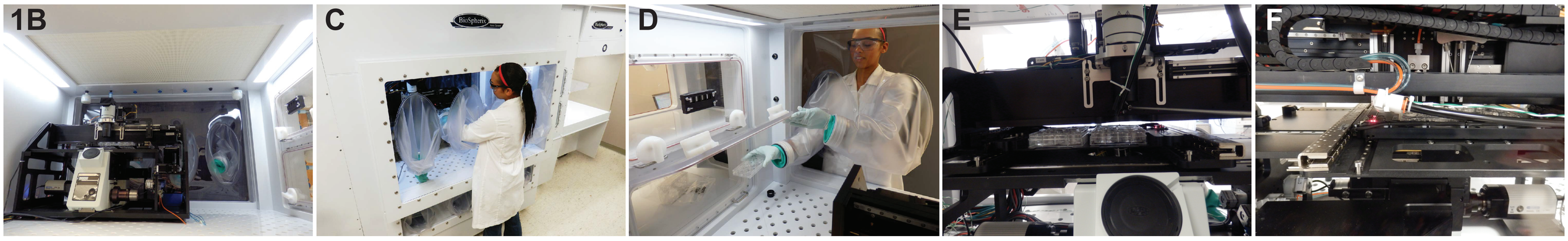


Figure 1. Experimental Design. (A) The Cell X™ Cell Automated Colony Picking System was enclosed in an Xvivo Cytocentric Barrier Isolator to test removal of airborne particles from the cellular environment. **(B)** HEPA filtered air was directed from above the Cell X downward through a ventilated floor. **(C)** Operation of the Cell X in the completely controlled atmosphere was accomplished using a soft glovefront. **(D)** To maintain the aseptic environment, chemically decontaminated materials were imported through a dual upper/lower buffer chambers which replaced any entering air with HEPA-filtered, tanked, medical-grade gases. To assess local particle levels, independent particle monitor sample intakes were positioned in the front **(E)** and rear **(F)** of the microscope stage.

Results

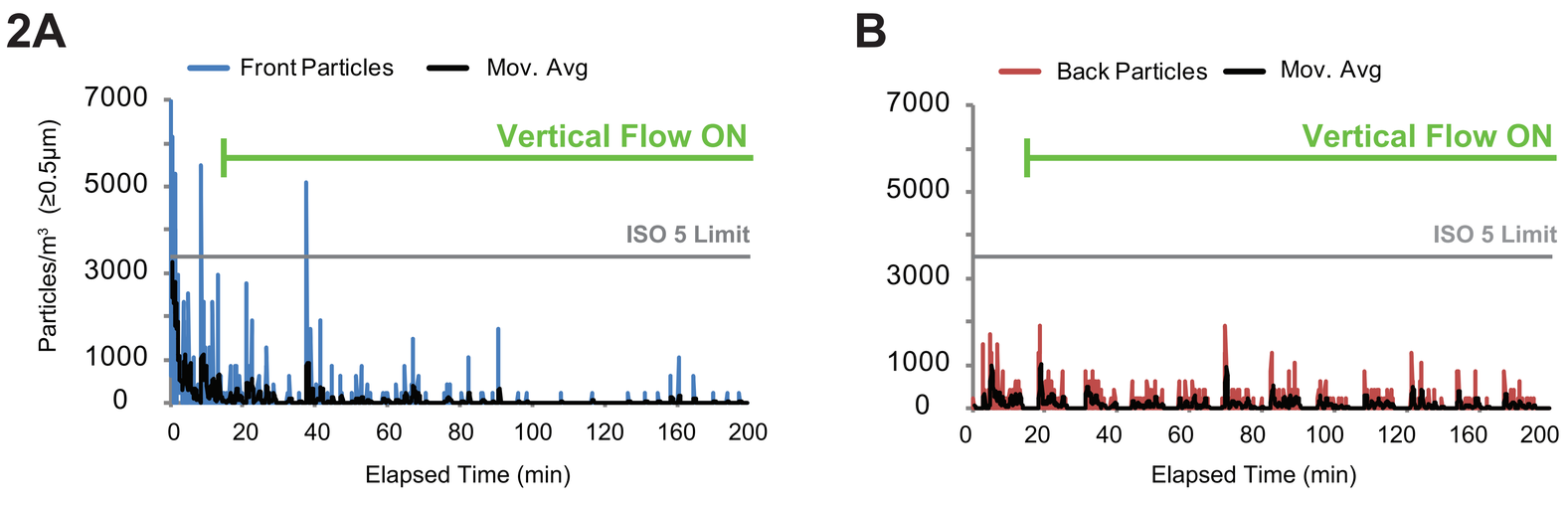


Figure 2. Experimental Design. (A) The Cell X automated colony picker was programmed for a prescribed set of motions, 12 repetitions for each set. Wtihout the Vertical Laminar Flow system in operation, particles spiked above the ISO 5 limit of 3500 particles/m^3 on the front particle sensor. With the Vertical Flow system on, particles were actively removed from the atmosphere, keeping particles below the ISO 5 limit except for one spike at about 38 minutes. At this point, the operator put their hands in the gloves and performed some simple manipulations.

Conclusions

- Cell culture-handling machinery generates airborne particles
- The Xvivo barrier isolator vertical flow system removes particles from the cell handling space, maintaining particle levels below ISO 5 limits

References

1. Ronn, R.E., et al., Reactive Oxygen Species Impair the Function of CD90+ Hematopoietic Progenitors Generated from Human Pluripotent Stem Cells. Stem Cells, 2017. 35(1): p. 197-206.

2. Kumar, A., et al., Quantifying the magnitude of the oxygen artefact inherent in culturing airway cells under atmospheric oxygen versus physiological levels. FEBS Lett, 2016. 590(2): p. 258-69.