Controlled Conditions Throughout Cell Handling Steps Increases Cell Culture Yields at Physiologically Relevant *in vitro* Oxygen Levels

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Abstract

Awareness of the sensitivity of induced pluripotent stem cells to oxygen has driven use of oxygen-controlled incubators for more physiologically relevant *in vitro* conditions. Oxygen levels have a profound effect on stem cell fate. The timescale of cellular responses to oxygen control disruptions makes cell handling steps performed in the HEPA-filtered room air of a standard biological safety cabinet a potential risk to consistent cell culture performance. Since Hypoxia-Induced Factor – alpha proteins are down-regulated at the protein level within minutes of higher oxygen exposure and take hours to return to active levels upon return to the incubator, we hypothesized that even brief disruption of optimal conditions during routine handling would affect cell culture expansion. We tested this hypothesis using K562, a multipotential hematopoietic cell line often used for T cell production process development like CAR-T cell production. K562 cultures were split into six identical flasks and their growth in 5% oxygen/5% carbon dioxide monitored for 14 days. Three cultures were housed in an external incubator fitted with an oxygen-controlled subchamber. Processing steps were performed in a HEPA-filtered room-air laminar flow hood (Room-Air Hood). The other three cultures were grown in an incubation chamber installed within a closed processing chamber (Hypoxia Hood), so that during all handling steps, conditions remained constant. All media for both groups were pre-equilibrated and pre-warmed before use. Statistically higher cumulative cell yields (p=0.003956, two-tailed T test, unequal variances) were seen under full-time oxygen control (Hypoxia Hood™) than in conditions that were broken briefly for routine cell sampling and passaging (Room-Air Hood). Cell viability was greater than 98% at all times in both groups, suggesting a cytostatic rather than cytotoxic effect on cell populations. We conclude that even without a highly visible effect on cell viability, cell cultures kept in physiologically relevant oxygen conditions are sensitive to brief disruptions in conditions. Full-time protection of cell cultures from room air, even during handling steps, may prevent detrimental long-term effects and increase *in vitro* cellular production yields.

Introduction

- Physiologic O_2 levels are lower than room air
- HIF-alpha protein levels change in seconds to minutes in response to room air exposure[1]
- Cells grown in low oxygen are out of optimum for hours after room air handling [2]
- Others have reported broken conditions as sufficient for cell growth [3]
- We tested the long-term effects of broken conditions on cell growth

Objectives

Compare the effects of room air laminar flow hood (broken) and Hypoxia Hood (unbroken) cell handling conditions on cell viability and yield over two weeks of routine cell culture at physiologically relevant oxygen levels.

Methods

- K562 cells were split into six flasks, three per group.
- One group was incubated in a standard CO_2 incubator equipped with a CO_2- and O_2-controlled subchamber, the other in a Hypoxia Hood which encloses an incubator and cell handling space in a single CO_2- and O_2- controlled unit.
- Cell counts and viability were assessed at twice-weekly subcultures by trypan blue exclusion.

Details:

K562 human leukemic cultures (ATCC Manassas, VA), in log-phase growth at 5% O_2, 5%CO_2 in RPMI-1640 medium (Invitrogen, Grand Island, NY) plus 10% FBS and glutamine (Sigma, St. Louis, MO) in a barrier isolator, were split into 6 T75 flasks (Celltreat, Shirley, MA). Three flasks were cultured in unbroken oxygen conditions, in the Hypoxia Hood for all incubation and handling, and three were cultured in an oxygen-controlled subchamber within a standard cell culture incubator and handled in a room-air laminar flow hood just long enough for cell sampling and routine cell culture twice weekly. Cells were counted and viability was assessed at each subculture using trypan blue exclusion.
Paired T-tests (Excel, Microsoft (Seattle, WA)) were used for comparisons.

References

1.Jewell, U.R., et al., Induction of HIF-1alpha in response to hypoxia is instantaneous. FASEB J, 2001. 15(7): p. 1312-4.
2.Allen, C.B., B.K. Schneider, and C.W. White, Limitations to oxygen diffusion and equilibration in in vitro cell exposure systems in hyperoxia and hypoxia. Am J Physiol Lung Cell Mol Physiol, 2001. 281(4): p. L1021-7.
3.Henderson, J.H., et al., Low oxygen tension during incubation periods of chondrocyte expansion is sufficient to enhance postexpansion chondrogenesis. Tissue Eng Part A, 2010. 16(5): p. 1585-93.

Methods (cont.)

A. Unbroken Conditions
Incubation 37°C, 5% O_2, 5%CO_2
Handling 37°C, 5% O_2, 5%CO_2

B. Broken Conditions
Incubation 37°C, 5% O_2, 5%CO_2
Handling 25°C, 20% O_2, 0.5%CO_2

Figure 1. Experimental Set-Up

(A) Three flasks K562 cells were maintained in the Hypoxia Hood for all incubation and handling. (B) Three were incubated in a standard CO_2 cell culture incubator containing an oxygen-controlled subchamber. These cultures were moved to a laminar flow hood for all handling steps during twice a week cell culture sessions over two weeks.

Results

- Cultures incubated and handled in unbroken conditions in the Hypoxia Hood had higher cell numbers than cells handled in room-air (broken) conditions.
- Cell viability was 98% or more in both groups at all times.

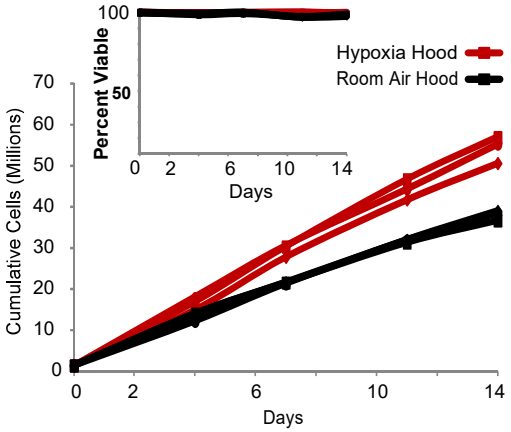

Figure 2. Cultures Handled in Room Air (Broken Oxygen Conditions) Lagged Cultures in Unbroken Conditions without Obvious Cytotoxicity

Cell growth at at 5% oxygen with cell handling either in the Hypoxia Hood or in a room-air laminar flow hood. There were statistically significant differences between the growth in the two conditions (p=0.003957, Day 14, two-tailed paired T test assuming unequal variances). Maintaining the cells inside the Hypoxia Hood for all incubation and handling produced larger cell yields over time, even though overt toxicity was not evident in the cells handled in room air (inset).

Conclusions

- **Unbroken conditions during cell handling increased cell yields.**
- **Viability in broken conditions was good even with reduced growth**
- **The negative effect of broken conditions during cell handling is not casually evident.**