Full-Time Controlled Conditions for Cell Therapy Production Environments Reduce Problematic Variability for Cells

Alicia D. Henn, Shannon Darou, Randy Yerden. BioSpherix, Ltd. Parish, NY

Abstract

Recent reports of variability in CAR-T cell products like Novartis' Kymriah have raised many questions about the influence of not only the individual patients' cells, but also the cellular environment upon cell product yield and phenotype. Temperature fluctuations have been identified as a source of variability in commercial cell processing. Here we examined the effect of room air conditions during routine twice weekly cell handling as a source of variability. We cultured cells in two different conditions: (1) normal room air incubator (37°C/ 5%CO_2/18%O_2) with handling of cells in BSC room air (25°C/ 0.02%CO_2/21%O_2) (2) constant physiologic conditions (37°C/ 5%CO_2/5%O_2) with the Xvivo System. We found better growth in cultures maintained in constant conditions that weren't subjected to changes in temperature and gas levels. This could have broad application to the consistency problems seen in the commercial cell production industry in that reducing environmental variability may further limit product variability to patient-specific sources.

Background

Recent reports have identified cell product variability as a serious and industry-wide challenge to cell therapy commercialization[1].

Changing environmental conditions can stress cells, affecting phenotype and function[2]. HIF-1a, a key controller of proliferation/differentiation, is regulated in as little as 5 min after oxygen changes[3].

Reducing variability in the cellular environment for commercial-scale cell cultures has been shown to reduce critical cell attribute variability[4].

The Xvivo System isolates the cell environment from variable room air providing constant incubator-like conditions for cells even during cell handling.

References

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2. Gropper, Y., et al., Culturing CTLs under Hypoxic Conditions Enhances Their Cytolysis and Improves Their Anti-tumor Function. Cell Rep, 2017. 20(11): p. 2547-2555.
3. Jewell, U.R., et al., Induction of HIF-1alpha in response to hypoxia is instantaneous. FASEB J, 2001. 15(7): p. 1312-4.
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Experimental Design

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Figure 1. Experimental Set-Up. Human cells were thawed under physiologic conditions (37°C, 5%CO2, 5%O2) and split into two conditions: (1) incubation in a traditional room air CO_2 incubator (37°C/ 5%CO_2/18%O_2) with cell handling in supraphysioxic room air (biological safety cabinet conditions) at each passage or medium change (25°C/ 0.02%CO_2/21%O_2) or (2) unbroken controlled conditions in the Xvivo System (37°C/ 5%CO_2/5%O_2). In this system, incubators (black doors) open only into a controlled cell handling space, allowing full-time control of the critical cell parameters including O_2, CO_2, humidity, and temperature. Three flasks of cells (Lonza Poietics human mesenchymal stromal cells) were incubated in each set of conditions. One flask of each set was set on a Lonza CytoSMART microscope to monitor cell coverage over time. Two additional flasks from each set were used to monitor cell culture growth with cell counts at biweekly passage. Cell culture medium was changed every 3-4 days even if cells did not need passaging to new flasks. Trypan blue exclusion was used for cell counts and results were assessed for statistical overlap between the groups using standard Excel spreadsheet software (Microsoft, USA).

Results

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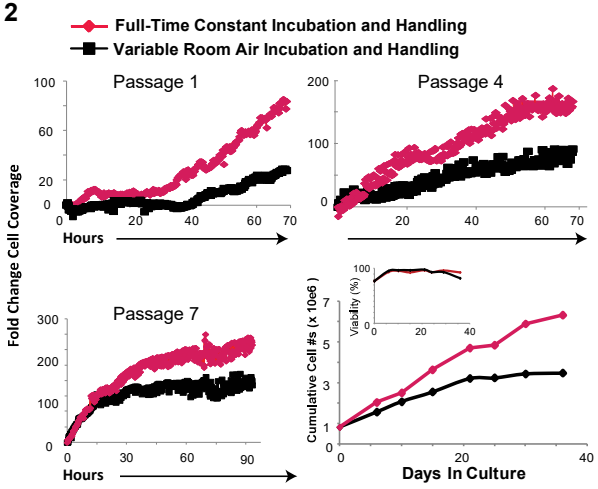

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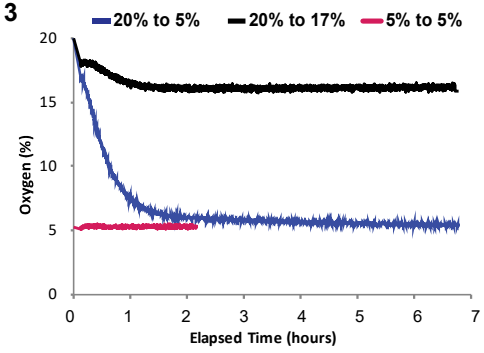

Figure 3. It takes Hours to Equilibrate Medium with Incubator O2. An O2 sensor was sealed through the wall of a T-25 flask and O2 levels recorded. It took over an hour for medium at room air O_2 (21%) to equilibrate to the lower room air incubator (17-18%) and almost 7 hrs for medium to equilbrate to physiologic 5% O_2. Medium under controlled conditions for cell handling at constant 5% O_2 and CO_2 took no time to equilibrate. It only takes 5 min for HIF-1a to be modulated, so hours-long equilibration times may affect cell proliferation and differentiation.

Figure 2.. Full-time Control of Cell Incubation and Handling Conditions Yielded Higher Cell Numbers and More Passages before Senescence. Variable standard room incubation and cell handling conditions reduced cell yields as compared to cells grown under full-time optimal conditions. Early, mid-, and late-passage cells all showed this effect by both time-lapse CytoSMART cell coverage densities assessments and trypan blue cell counts. Cell viability was high for both conditions (inset) until late passage. This effect on cell growth would go unnoticed if not actively comparing conditions. A paired T-test (2-tailed) showed unlikely statistical overlap between the cell counts of the two groups at each passage (p=0.0079).

Conclusions

Full-time control of all cellular conditions including temperature and gas levels can reduce process variability for cells and improve cell culture growth