PHYSIOLOGICALLY RELEVANT OXYGEN DURING CELL HANDLING AS WELL AS INCUBATION ENHANCES THE GROWTH OF HUMAN MESENCHYMAL STEM CELLS

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

Abstract

Reproducible research on any physiologic or pathophysiologic state requires relevant in vitro conditions for cells. It is well-known that when cultured at room oxygen levels, clinically important Mesenchymal Stem/ Stromal Cells (MSC), experience oxidative stress. However, there is a common opinion that if cultured at physiologic oxygen, brief exposures to room air during cell handling won't affect the cells. So we tested the hypothesis that even if incubated at physiologically relevant oxygen levels, human bone marrow MSC exposed to room air during routine cell handling would have equivalent growth characteristics as MSC maintained in unbroken physiologic conditions. Human MSC cultures were cultured in T flasks in 5% O2/5% CO2. Three cultures were housed in an external incubator fitted with an oxygen-controlled subchamber and handled in HEPA-filtered room air conditions (Room-Air). The other three cultures were housed within a closed processing chamber (Hypoxia Hood) that provided constant conditions for handling as well as incubation. We also measured pericellular oxygen levels with an oxygen probe. Cellular growth was monitored using the CytoSMART live cell imaging system which provided constant monitoring without disturbing the cells. Cell culture media were pre-equilibrated to the matching cell handling conditions before use. Pericellular oxygen levels in room-air handled flasks took over an hour to equilibrate to the incubator levels. Cell viability was greater than 90% at all times in both groups, but our data did not support our hypothesis. Statistically higher cell yields were seen when cells were handled under full-time oxygen and CO_2 control (Hypoxia Hood) than in Room-Air cell handling conditions. The cells also stayed in active cell growth for more passages. Further experiments showed that full-time optimal conditions produced higher cell yields than traditional cell conditions with no oxygen control. We conclude that breaking physiologic conditions for routine cell handling had a detrimental effect on MSC growth. This is not an effect that can be easily seen by the cell culturist as cytotoxic effect, but was a cytostatic effect. Maintaining full-time physiologic gas levels during cell handling as well as incubation is beneficial for physiologically relevant research of clinically important MSC.

Introduction

- Human mesenchymal stem/stromal cells (MSC) are of extremely high clinical value. Optimization of *in vitro* expansion methods is critical[1]
- MSC originate from tissues with low O_2 levels
- HIF-1a is affected within 5 minutes of O_2 change[2]
- Supraphysiologic and variable oxygen levels found in traditional cell culture and handling settings are highly stressful for MSC[3] and can affect proliferation[4]

Objective

Using the Hypoxia Hood for unbroken atmospheric control and the CytoSMART for unbroken observations, compare MSC growth under:

A. Full-Time Optimal Conditions for Incubation and Handling: Physiologic Temp, CO_2 and O_2

B. Optimal Incubation, Sub-optimal Handling (Room temperature, hypocapnic, hyperoxic)

C. Traditional Sub-Optimal Conditions for Incubation and Handling

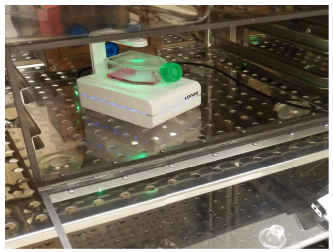
References

1.Hoch, A.I. and J.K. Leach, Concise Review: Optimizing Expansion of Bone Marrow Mesenchymal Stem/Stromal Cells for Clinical Applications. Stem Cells Translational Medicine, 2015. 4(4): p. 412-412.
2.Jewell, U.R., et al., Induction of HIF-1alpha in response to hypoxia is instantaneous. FASEB J, 2001. 15(7): p. 1312-4.
3.Phinney, D.G., et al., Mesenchymal stem cells use extracellular vesicles to outsource mitophagy and shuttle microRNAs. Nat Commun, 2015. 6: p. 8472.
4.Boregowda, S.V., et al., A Clinical Indications Prediction Scale Based on TWIST1 for Human Mesenchymal Stem Cells. EBioMedicine, 2016. 4: p. 62-73.

Methods

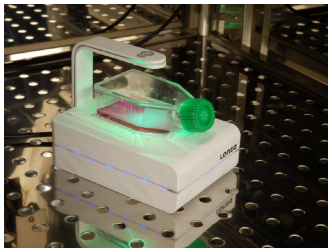
A. Full-Time Optimal Conditions

	°C	%CO_2	%O_2
Incubation	37	5	5
Handling	37	5	5



B. Part-Time Optimal

	Incubator	Handling (BSC)
°C	37	25
%CO_2	5	0.05
%O_2	5	20



C. Traditional Sub-Optimal

	Incubator	Handling (BSC)
°C	37	25
%CO_2	5	0.05
%O_2	16-19	20

Figure 1. Experimental Set-Up. Lonza Poietics human bone marrow MSC cells were grown with Lonza hMSC Basal Medium plus MSCGM, hMSC Singlequot Kit, and Lonza Trypsin/EDTA for MSC, and Lonza DPBS w/o Ca/Mg (Lonza, Cologne, GmbH). Cells were thawed under physiologic conditions (37°C, 5%CO2, 5%O2) and immediately split into two conditions per run, either conditions A and B or conditions A and C in triplicate. One flask of each set was used for monitoring with the Lonza CytoSMART microscope and two flasks were used to pool with the first for cell counts at each passage. Cells were monitored for cell density and the subcultured when no more than 80% confluent. Medium was changed every 3-4 days even if cells did not need passaging. Cells were trypsinized, washed, and counted at each passage. Cell coverage data and time-lapse photomicrographs were provided by the CytoSMART. Cell counts were assessed for statistical overlap using Excel (Microsoft, USA).

Results

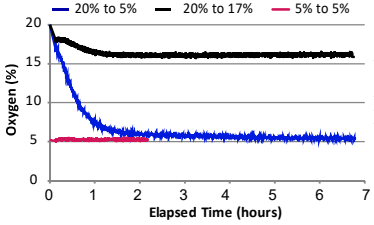


Figure 2. Time to Equilibrate Medium with Incubator O2. An O2 sensor probe was placed through a hole in the wall of a T-25 flask and O2 levels recorded. It took almost 7 hours for medium pre-equilibrated to 20% O2 to completely equilbrate with 5% O2 in the chamber. It took over an hour for medium equilibrated to 20% O2 to equilibrate with O2 in a traditional room air incubator (17%) (blue). Medium pre-equilibrated to 5% O2 took no time to equilibrate with the 5% O2 atmosphere

Results (cont'd)

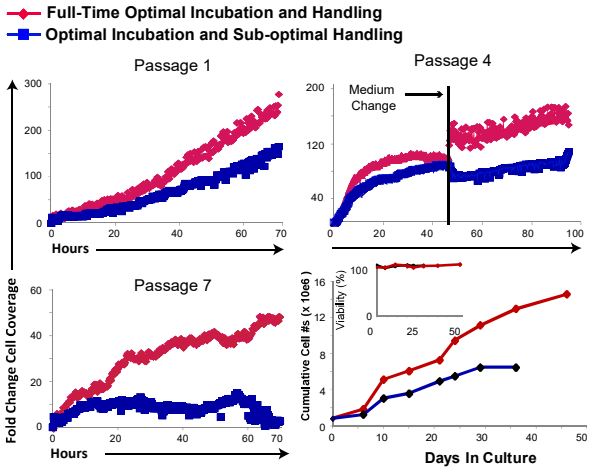


Figure 3. Differential Effects of Full-Time Optimal and Part-Time Optimal Cell Culture Conditions on MSC. MSC grown under full-time optimal conditions for both incubation and handling consistently grew to higher yields than cells exposed to sub-optimal room air conditions for routine passage. This was consistent in early, mid-, and late-passage cultures, as assessed by CytoSMART cell coverage and by trypan blue cell counts. Cells in both conditions had good viability (inset), suggesting a cytostatic effect rather than a cytotoxic effect. Higher overall cumulative cell numbers were evident as well as more passages in unbroken optimal conditions. A 2-tailed paired T-test showed unlikely statistical overlap of yield at each passage (p=0.0079).

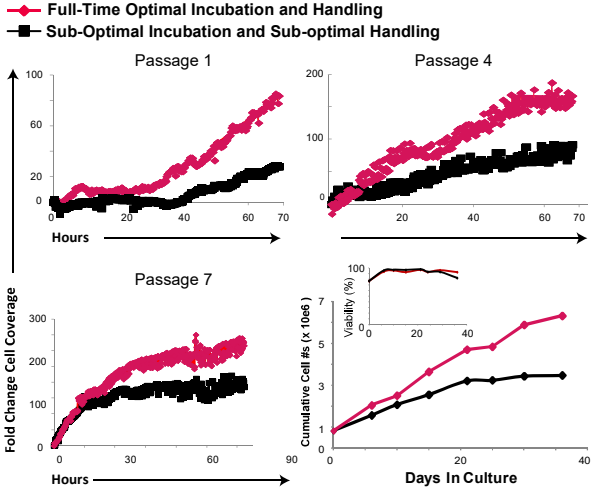


Figure 4. Differential Effects of Full-Time Optimal and Traditional Sub-Optimal Room Air Cell Culture Conditions on MSC. MSC grown under traditional cell culture conditions (no oxygen control during incubation, HEPA-filtered room air conditions for cell handling) did not yield as high cell numbers as cells grown under full-time optimal conditions. Early, mid-, and late- passage cells all showed this effect by both CytoSMART cell densities and trypan blue counts. Cell viability was high for both conditions (inset), it seems again, to be an effect that would go unnoticed if a researcher was not actively comparing conditions. A paired T-test (2-tailed) showed unlikely statistical overlap between the groups at each passage (p=0.0079).

Conclusions

MSC are exposed to sub-optimal oxygen levels for hours after handling in sub-optimal conditions.

Full-time control of oxygen for both incubation and cell handling increases MSC yields over part-time optimal or traditional conditions.