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INCREASING HUMAN MSC YIELDS THROUGH UNBROKEN PHYSIOXIA

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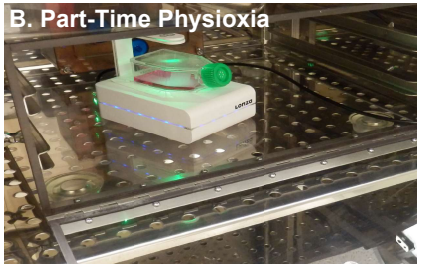
Abstract

There is a large body of literature that documents the stresses that supraphysioxic conditions place on human bone marrow mesenchymal stromal/stem cells (MSC), yet traditional room air culture practices with these clinically relevant cells still persist. We tested the hypothesis that MSC provided with physioxia (3 – 5% O2) just for incubation, and then handled in the supraphysioxia of room air (as in a biological safety cabinet), would have equivalent growth characteristics as MSC maintained in unbroken physioxia and CO2. Human bone marrow MSC cultured in triplicate T-75 flasks of MSC were housed for up to 8 passages in an external incubator fitted with an oxygen-controlled subchamber set to 5% O2/5% CO2. They were placed in HEPA-filtered room air conditions (Room-Air) for cell handling. The other set of cultures were housed within a closed processing chamber (Xvivo System) for full-time control of oxygen and CO2. Cell culture media were pre-equilibrated to the matching cell handling conditions before use. Cell growth was recorded using the CytoSMART cell imaging system. We found that the two sets of conditions did not produce equivalent cell growth. There were higher yields in each passage, and more total cell passages in cultures maintained full-time in physioxic conditions than in cultures handled under traditional supraphysioxic conditions (two-tailed T test, unequal variances). We went on to look at how long it took pericellular and intracellular oxygen levels to recover after a quick room air medium change with medium that was not pre-equilibrated to physioxic levels. We measured pericellular oxygen levels with an oxygen probe and intracellular oxygen with an intracellular oxygen indicator dye. Pericellular oxygen levels in cultures handled in room air took over 80 minutes to equilibrate to the physioxia, far longer than it takes to modulate HIF-1a levels in MSC. Intracellular oxygen took even longer to recover, over 150 minutes. Therefore, controlling oxygen levels around MSC during cell handling operations is critical to maximizing MSC yields both for research and clinical applications.

Background

- Human mesenchymal stem/stromal cells (MSC) are of extremely high clinical value[2].
- MSC originate from tissues with low O_2 levels and tend to use aerobic glycolosis[1,5].
- HIF-1a is affected within 5 minutes of O_2 change[3]
- MSC are negatively impacted by increased ROS in room air culture[5].
- Supraphysiologic and variable oxygen levels found in traditional cell culture and handling settings are highly stressful for MSC[1] and can affect proliferation[2,4]

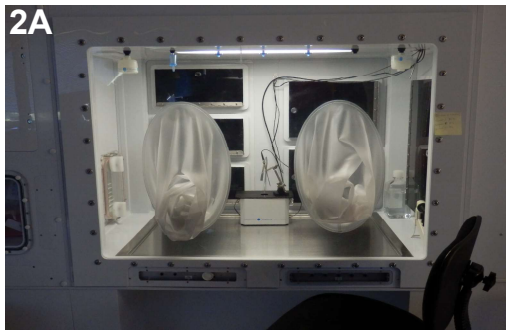
Methods



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

	°C	%CO_2	%O_2
Incubation	37	5	5
Handling	25	0.1	20

Figure 1. 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, A and B. 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 then 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).



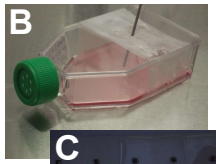


Figure 2. Human bone marrow MSC (Lonza Gmb) were maintained in 3%O_2 5% CO_2 conditions for handling as well as incubation (A) in the Xvivo System. Incubators (black doors) open into a closed cell handling space with a heated floor and an atmosphere from tanked, filtered gases. To mimic the conditions in which cells are passaged in room air and returned to a tri-gas incubator, MSC were passaged at 20% O_2 plus 5%CO_2 and an immersion probe used to measure O_2 levels in the flask headspace and medium as the chamber was equilibated to 3%O_2. For intracellular O_2, the MitoXpress Intra Intracellular Oxygen Assay (Agilent) was used. MSC grown at 3%O_2 were plated at three densities and loaded with the Intra reagent overnight. The next day, the cells were washed and the intracellular oxygen assays performed in the Xvivo System with an enclosed BMG plate reader.

Results

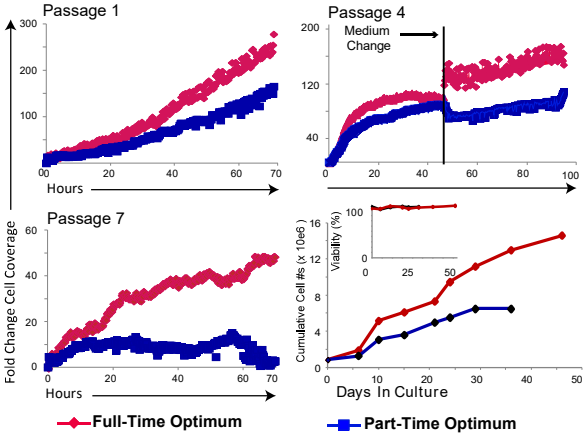


Figure 1. Differential Effects of Full-Time Optimal and Part-Time Optimal Cell Culture Conditions on MSC. MSC grown under full-time optimal conditions for both incubation and handling consistently grew to higher yields than cells exposed to sub-optimal room air conditions for routine passage. This was 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).

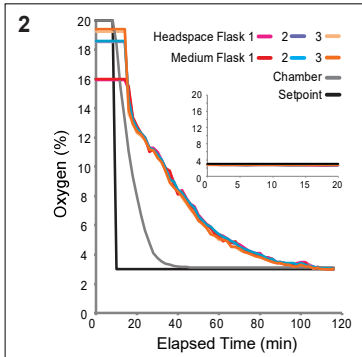


Figure 2. It Takes Almost Two Hours for a Vented T-75 Flask to Return to 3% O_2 After Passage in Uncontrolled Room Air Oxygen. Headspace and medium O_2 levels lagged far behind the chamber O_2 during equilibration in a T-75. A flask of cells returned to a tri-gas incubator does not equilibrate to chamber O_2 for hours. (Inset) Headspace and medium O_2 did not change when cells were handled under constant 3% O_2 and 5% CO_2.

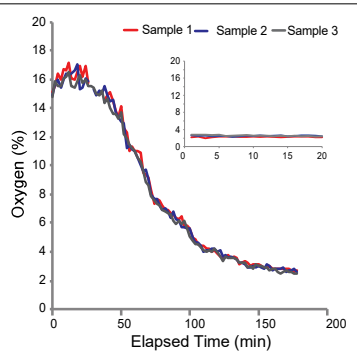


Figure 3. Cell Handling in Constant Conditions Eliminated Variability in Intracellular Oxygen. Intracellular O_2 levels were measured using Agilent's MitoXpress Intra Intracellular Oxygen Assay after a routine medium change at room air O_2 levels or at controlled 3% O_2. Inside the cell, O_2 levels take hours to recover after an exposure to room air for routine medium changes. (Inset) Changing medium in a chamber with controlled O_2 and CO_2 conditions eliminated this variation.

References

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Conclusions

- **Cell handling in room air O2 produces out-of-optimum pericellular and intracellular conditions for hours after cells are back in the incubator. Cell handling in full-time controlled conditions prevents these variations.**
- **Full-time control of oxygen for incubation and cell handling increases MSC yields over part-time optimal conditions.**