Physioxic Control of Cell Handling Conditions Reduces Variability for Human MSC

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Abstract

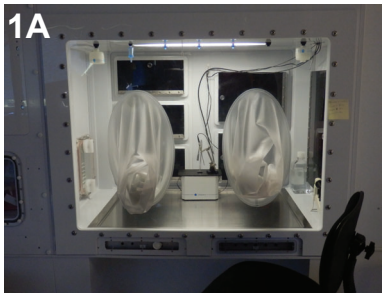
The importance of incubating clinically valuable mesenchymal stem/stromal cells (MSC) at low oxgyen levels has been well established, but many cell culturists still handle MSC in conventional room air biological safety cabinets (BSC). This practice causes stress in MSC as they experience swings in conditions and increasing experimental variability. MSC utilize aerobic glycolysis which consumes less O_2 than oxphos even when O_2 is abundant. We have previously published that room air cell handling reduces MSC yields and speeds culture senscence. For this project, we sought to establish how long it takes pericellular O_2 to return to optimum low levels after MSC undergo routine passaging in a room air atmosphere. Using the Xvivo System, in which we control gas levels and temperature for the cells during all cell handling steps as well as incubation, we sealed an immersion oxygen probe into a vented-cap T-75 flask of human bone marrow MSC cells immediately after passage. We kept the flask in the cell handling chamber, which was at 37ºC and 20% O_2, and set the atmosphere to 3% O_2. We monitored pericellular O_2 levels as the chamber atmosphere, the vessel headspace, and the cell culture medium equilibrated to 3% O_2. With 15ml of medium sitting undisturbed, we found that it took between 1 and 2 hours for pericellualr O_2 levels to equilibrate to chamber oxygen levels. T his is a long period of time for MSC to be in stress-inducing supra-physiologic oxygen levels. When cell culture medium was pre-equilibrated and conditions controlled, the cells stayed at optimum levels. We concluded that full-time control of cell handling atmosphere is necessary to reduce variability in conditions for MSC.

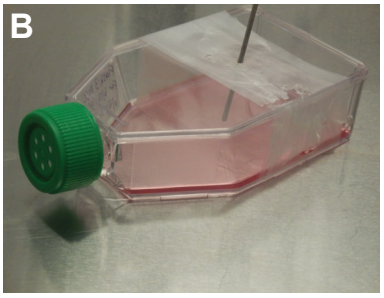
Background

Room air oxygen[1] and changes in oxygen levels[2] are stressful to MSC and can affect MSC function[3].
Human MSC tend to use aerobic glycolysis[3], and are negatively impacted by increased ROS in room air culture[1].

Null Hypothesis: Cells are not out of optimum for more than the 5 min it takes to change medium in a room air BSC and return them to a tri-gas incubator

Experimental Design





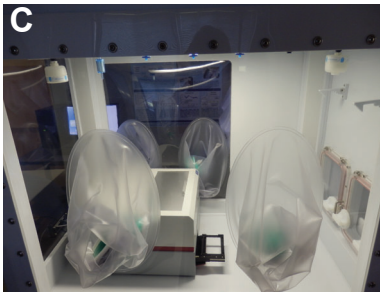


Figure 1. Experimental Design. Human bone marrow MSC (Lonza Gmb) were maintained in 3%O_2 5% CO_2 conditions for handling as well as incubation (A) in the Xvivo System. Incubators (black doors) open into a closed cell handling space with a heated floor and an atmosphere from tanked, filtered gases. To 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

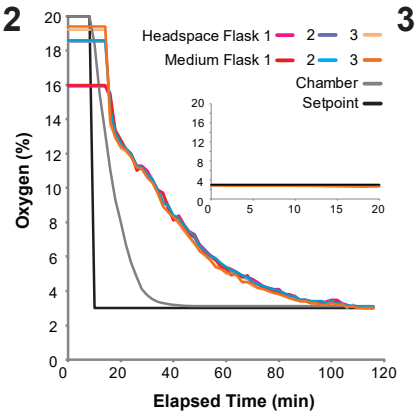


Figure 2. It Takes Almost Two Hours for a Vented T-75 Flask Conditions to Return to 3% O_2 After Passage in Uncontrolled Room Air Oxygen. Headspace and medium O_2 levels lagged far behind the chamber O_2 during equilibration in a T-75. A flask of cells returned to a tri-gas incubator does not equilibrate 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

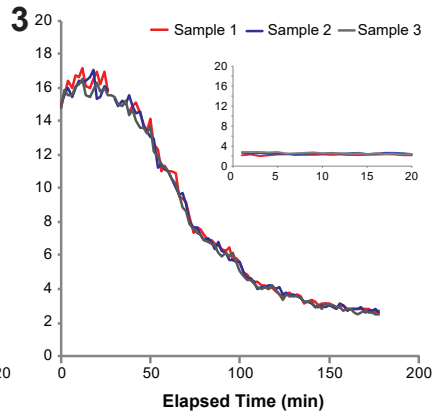


Figure 3. Cell Handling in Constant Conditions Eliminated Variability in Intracellular Oxygen. Intracellular O_2 levels were measured using Agilent's MitoXpress Intra Intracellular Oxygen Assay after a routine medium change at room air oxygen levels or at a controlled 3% O_2. Inside the cell, O_2 levels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

Conclusions

Hypothesis disproved. Cell handling in room air O_2 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 out-of-optimum variations in conditions.

References

1. Boregowda, S. V., Krishnappa, V., Chambers, J. W., Lograsso, P. V., Lai, W. T., Ortiz, L. A., & Phinney, D. G. (2012). Atmospheric oxygen inhibits growth and differentiation of marrow-derived mouse mesenchymal stem cells via a p53-dependent mechanism: implications for long-term culture expansion. Stem Cells, 30(5), 975-987. doi:10.1002/stem.1069
2. Pattappa, G., Thorpe, S. D., Jegard, N. C., Heywood, H. K., de Bruijn, J. D., & Lee, D. A. (2013). Continuous and uninterrupted oxygen tension influences the colony formation and oxidative metabolism of human mesenchymal stem cells. Tissue Eng Part C Methods, 19(1), 68-79. doi:10.1089/ten.TEC.2011.0734
3. Paquet, J., Deschepper, M., Moya, A., Logeart-Avramoglou, D., Boisson-Vidal, C., & Petite, H. (2015). Oxygen Tension Regulates Human Mesenchymal Stem Cell Paracrine Functions. Stem Cells Transl Med, 4(7), 809-821. doi:10.5966/sctm.2014-0180
4. Boshoff, C. (2007). Transformation of human mesenchymal stem cells increases their dependency on oxidative phosphorylation for energy production. Proceedings of the National Academy of Sciences, 104(15), 6223-6228. doi:10.1073/pnas.0700690104