SHOCK FROM EXPOSURE TO ROOM AIR CONDITIONS ALTERS INDIVIDUAL MESENCHYMAL STEM CELL FATE, POPULATION DYNAMICS, AND BATCH YIELDS

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Abstract

Mesenchymal Stem/Stromal Cells (MSC) have become incredibly valuable, clinically and monetarily. Getting higher cell yields for cellular therapies can be critical to the success or failure of a cellular therapeutic product. MSC experience oxygen shock in non-physiologic room conditions. Even researchers that provide optimal physiologic conditions for their cells in the incubator, thinking that because the cells are exposed for only a short time, handle their cells in a HEPA-filtered room air BSC. However, HIF-alpha protein levels can change within 5 minutes of oxygen changes, affecting cell fate decisions by individual cells. We reported previously that pericellular oxygen levels in room-air handled cultures take hours to equilibrate to the incubator levels and that human bone marrow MSC exposed to room air during routine cell handling have lower cell yields. For the current studies, we hypothesized that those lower cell yields are due to altered cell fate at the individual cell levels and that these combined cell fate decisions contribute collectively to altered population dynamics over time. Human MSC cultures were cultured in T flasks in different conditions; Part-time controlled \conditions (5% O2/5% CO2 with an incubator sub chamber, room air conditions for handling), Full-time physiologic O2 and CO2 for both incubation and handling (in a Hypoxia Hood). The CytoSMART live cell imaging system provided time-lapse photomicrographs inside the incubators for constant monitoring without disturbing the cells. Pre- equilibrium of cell culture media assured appropriat e gas levels in solution before cells were exposed to fresh media. Cell viability was greater than 90% at all times in all groups. Individual time-lapse photo-micrographs were analyzed for cell fate decision on a cell-by-cell basis. Statistically higher numbers of mitotic events took place when cells were handled under full-time control of conditions (Hypoxia Hood) than in part-time control of conditions. Changing cell conditions during cell handling in room air induced a perturbation in temporal population growth patterns by changing the individual cell fate decisions. This effect is not one that can easily be seen during routine culture as cytotoxic effect, but was a cytostatic effect. We conclude that protecting cells from room-air full-time, during cell handling as well as incubation, is critical for promoting individual cell fate decisions that lead to optimal population growth and higher MSC yields for cellular therapies.

Introduction

- **Mesenchymal Stem/Stromal Cells (MSC) are of extremely high clinical value. Optimization of physiologic conditions is critical for higher cell yields**
- **MSC originate from tissues with low O2 levels**
- **HIF-alpha protein levels can change within 5 min of O2 changes, affecting cell fate decisions by individual cells**
- **Full-time physiologic conditions of O2 and CO2 for both incubation and handling are essential for cell yields**

Objectives

Using the Hypoxia hood and the CytoSMART for continuous observations, compare MSC growth under:

1. Part-time controlled conditions (5% O2/5% CO2 with an incubator sub chamber, room air conditions for handling)

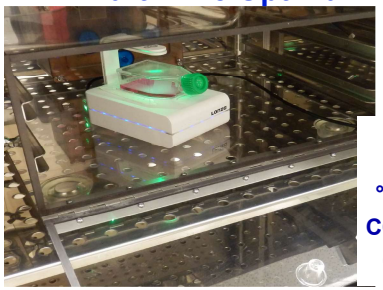
2. Full-time physiologic O2 and CO2 for both incubation and handling (in a Hypoxia Hood)

References

S.H., et al., Isolation and large-scale expansion of bone marrow-derived mesenchymal stem cells
erum-free media under GMP-compliance. mortality, 2014. 40: p. 1.
athe, C.S., et al., Islet cell transplantation in Australia: screening, remote transplantation, and incretin
ne secretion in insulin independent patients. Horm Metab Res, 2015. 47(1): p. 16-23.
l, T., P. Carson, and V. Falanga, Topical Delivery of Cultured Stem Cells to Human Non-Healing

Methods

A. Part-Time Optimal Conditions



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

B. Full-Time Optimal Conditions

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

Figure 1. Experimental Set-Up. Lonza human bone marrow MSC cells were thawed under physiologic conditions (37°C, 5%CO2, 5%O2) and immediately split into two conditions 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). One flask of each set was used for monitoring with the Lonza CytoSMART microscope and three were used for cell counts. Cells were monitored for cell density and the subcultured when no more than 80% confluent. Medium was exchanged every 3-4 days Cells were trypsinized, washed, and counted at each passage. Cell coverage data and time-lapse photomicrographs were provided by the CytoSMART. Statistical overlap between group cell counts were assessed using Excel (Microsoft, USA).

Results

A. Early Passage

O_2	Start Amount	Start Avg.	End Amount	End Avg.	Total New	Adherent By	Cells w/ no Mitosis	First Mitotic Event	Peak Mitosis Time	# of Cells Moved
Part-Time	205	17.08	248	20.67	43	6.5 hours	67%	7 hrs	32 hrs	3
Full-Time	159	13.25	239	19.92	80	8.5 hours	38%	2.75 hrs	26.5 hrs	1

B. Late Passage

O_2	Start Amount	Start Avg.	End Amount	End Avg.	Total New	Adherent By	Cells w/ no Mitosis	First Mitotic Event	Peak Mitosis Time	# of Cells Moved
Part-Time	113	9.42	158	13.17	45	5.5 hrs	46%	7 hrs	29 hrs	7
Full-Time	134	11.17	231	19.25	97	3.25 hrs	23%	7.25 hrs	29.25 hrs	2

Table 1. Cell Fate Analysis. After keeping cells in unbroken/broken conditions for approximately 3 days, the videos recorded by CytoSMART, as well as the first frame of each video, was downloaded. Grids (3x4) were then put on each separate frame. After each individual cell movement and mitotic event was recorded, the start and end amount of cells was compared. The mean was then taken of the amount of cells per box for starting and end points and was compared for part-time vs. full-time conditions; as well as the total number of cells created. Other comparisons were made including: the amount of time it took for cells to settle and become adherent, the percent of cells that did not go through mitosis, how long it took for the first cell to go through mitosis, the peak time of mitosis, and the number of cells that moved out of original box.

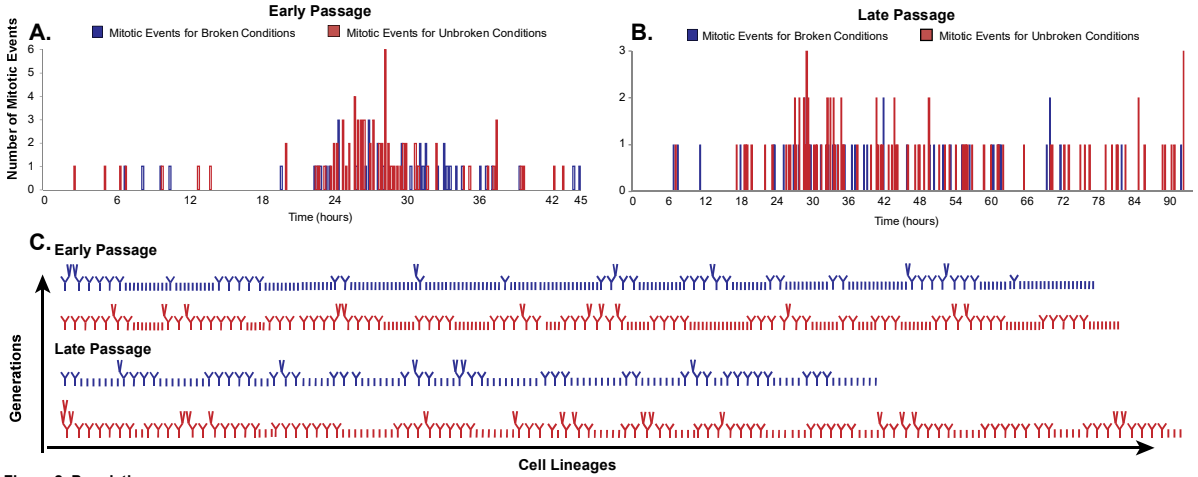


Figure 2. Population (A, B) Analysis of two sets of videos of part-time comparing mitotic events per 15 mins between unbroken conditions and broken conditions. A pattern is easily recognizable on both figures for a pause of events until after 6 hrs, and a peak of mitotic events around the 24th hour the cells have been left to incubate. C.also show that overall, unbroken conditions were able to create more cells per 15 minutes than broken conditions. Each line represents an individual cell in this batch; the "Y" formations represent a mitotic event, a division of that cell into two new, identical cells. Figures 8-9 show the comparison of how many individual cells divided, and how many times they divided, if at all. Again, a pattern is easily recognizable throughout the different batches of cells. The unbroken graphs show that most cells went through mitosis at least once, making their graphs longer. The broken graphs show that less cells divided, but divided longer. These patterns show that the unbroken conditions give the cells a more equal chance for all of them to be able to go through mitosis and divide; the broken conditions however, are more "survival of the fittest." The stronger cells are able to reproduce and keep their family tree growing longer, whereas the weaker cells are not able to reproduce. Overall, it shows that cells are more likely to divide within unbroken conditions, rather than broken ones.

Conclusion

Full-Time oxygen control doubled cell yield by protecting the ability of individual cells to divide