

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- α 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) and Full-time physiologic O₂ and CO₂ for both incubation and handling (in an Xvivo System). The CytoSMART live cell imaging system provided time-lapse photomicrographs inside the incubators for constant monitoring without disturbing the cells. Pre-equilibration of cell culture media assured appropriate 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 (Xvivo System) 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) originate from tissues with low O₂ levels
- HIF- α protein levels can change within 5 min of O₂ changes, affecting cell fate decisions by individual cells [1]
- Room Air O₂ levels are highly stressful for human MSC [2]
- Exposure to ROS affects cell cycle regulatory molecules [3]
- Previous studies from our group showed lower cell yields when physiologic culture conditions were interrupted for routine cell handling in room air.

Objectives

To determine if during cell recovery from room air exposure during cell handling, there is: a delay in mitosis, a decrease in number of generations, or a decrease in number of cells dividing.

Compare MSC growth under:

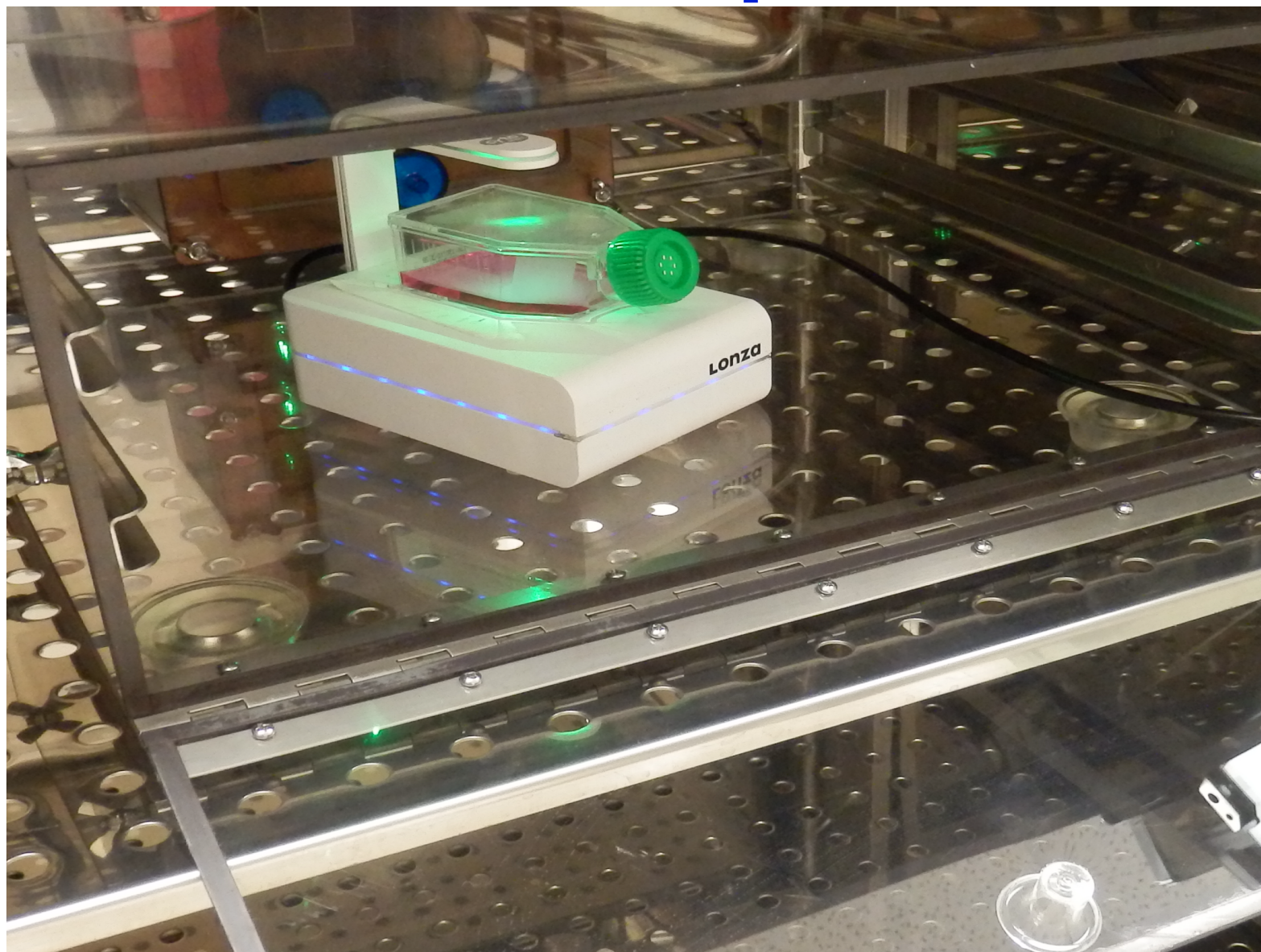
1. PART-time controlled conditions controlled for incubation, room air for handling
2. FULL-time physiologic O₂ and CO₂ incubation and handling (Xvivo X3 System)

References

1. Jewell, U.R., et al., Induction of HIF-1 α in response to hypoxia is instantaneous. FASEB J, 2001. 15(7): p. 1312-4.
2. Phinney, D.G., et al., Mesenchymal stem cells use extracellular vesicles to outsource mitophagy and shuttle microRNAs. Nat Commun, 2015. 6: p. 8472.
3. Burhans, W.C. and N.H. Heintz, The cell cycle is a redox cycle: linking phase-specific targets to cell fate. Free Radic Biol Med, 2009. 47(9): p. 1282-93.

Methods

A. Part-Time Optimal



	°C	CO ₂	O ₂
Incubation	37	5%	5%
Handling (BSC)	25	0.05%	20%

B. Full-Time Optimal Conditions



	°C	CO ₂	O ₂
Incubation	37	5%	5%
Handling	37	5%	5%

Figure 1. Experimental Set-Up.

Lonza Poietics human bone marrow MSC were thawed under physiologic conditions (37°C, 5%CO₂, 5%O₂) and immediately split into two conditions, part-time control with an incubator-housed oxygen controlled sub-chamber or full-time control with the Xvivo System. 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 pooled for cell counts. Cells were monitored for cell density and 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 System. Statistical overlap between group cell counts were assessed using Excel (Microsoft, USA).

Results

A.

Passage 1 After Thaw (P3 total)

	Total Hours	Adherent By (hrs)	# Cells migrated	Time to First Division (hr)	Starting Cell #	Ending Cell #	Fold Increase	# Cells Divided at Least Once	# Cells with No Cell Division
Part-Time	45	6.5	3	7	208	248	1.2	40	169
Full-Time	45	8.5	1	2.75	166	239	1.5	76	90

B.

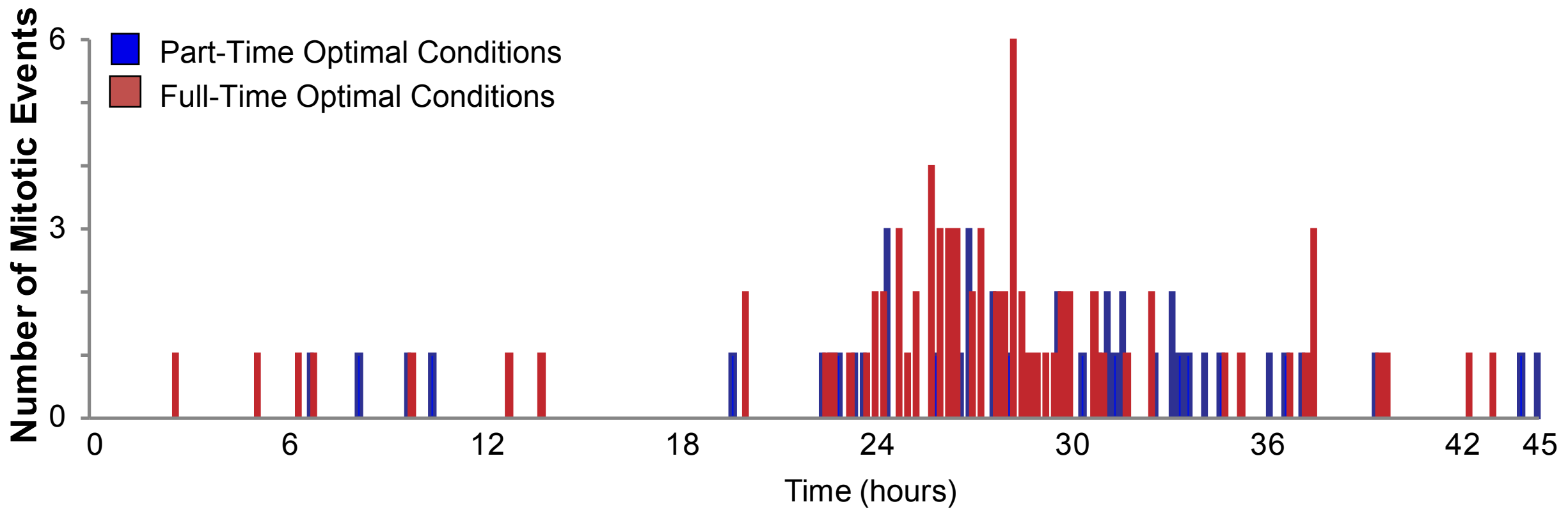
Passage 4 After Thaw (P6 Total)

	Total Hours	Adherent By	# Cells migrated	Time to First Division (hr)	Starting Cell #	Ending Cell #	Fold Increase	# Cells Divided at Least Once	# Cells with No Cell Division
Part-Time	96	5.5	7	7 hrs	113	158	1.4	44	73
Full-Time	96	3.25	2	7.25 hrs	134	231	1.7	89	37

Table 1. Cell Fate Analysis: Images recorded every 15 minutes over an entire cell passage by the Lonza CytoSMART were downloaded after culture at early passage (A) or later passage (B). Grids (3x4) were superimposed on each frame. The cells in each frame in the initial image were labelled, and their behavior over time was recorded, including any cell division, any migration of the cell out of the starting frame and the amount of time for all cells to adhere after re-seeding. We found there were no consistent differences in time to adhere or first cell division. However, the number of cells that divided at all were consistently higher in cells not exposed to room air conditions.

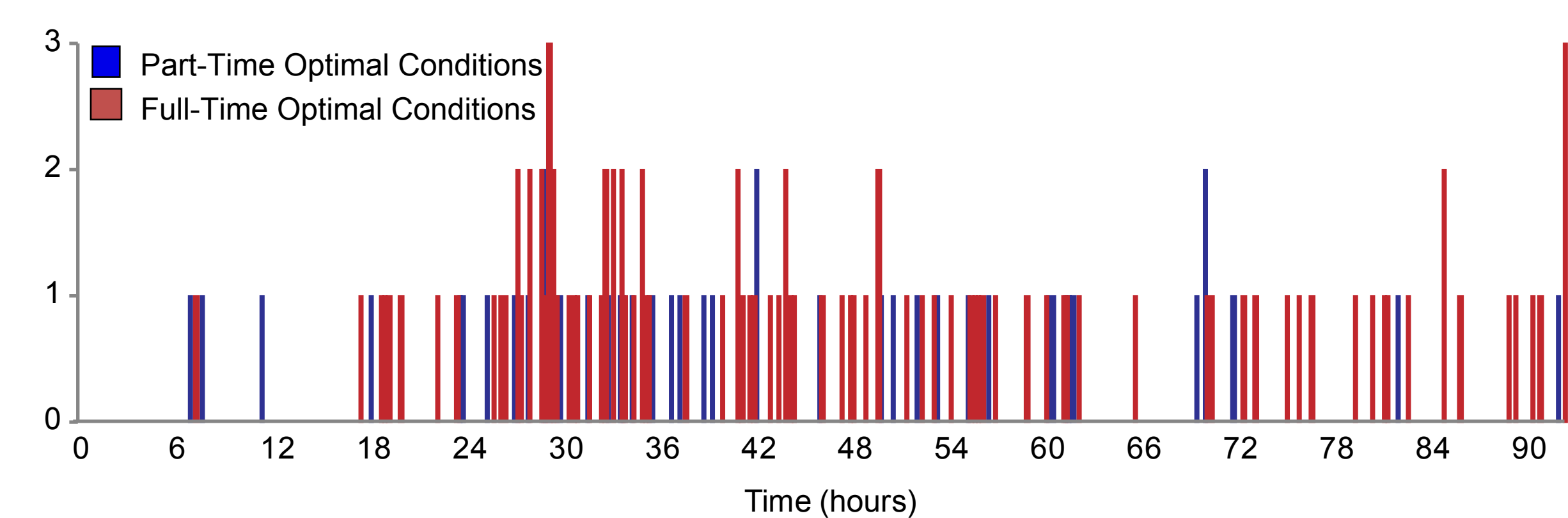
A.

Mitotic Events - Early Passage



B.

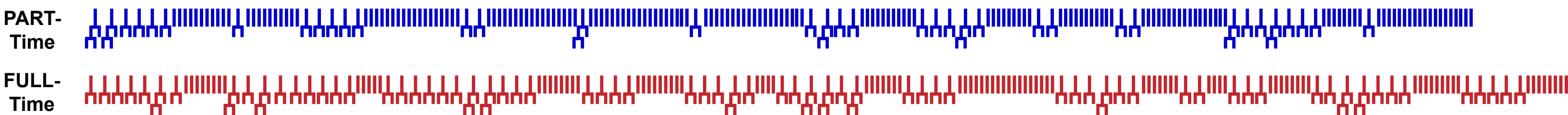
Mitotic Events - Late Passage



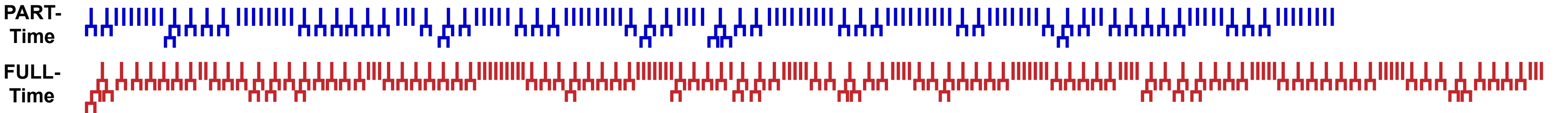
C.

Individual Cell Lineages

Early Passage



Late Passage



D.

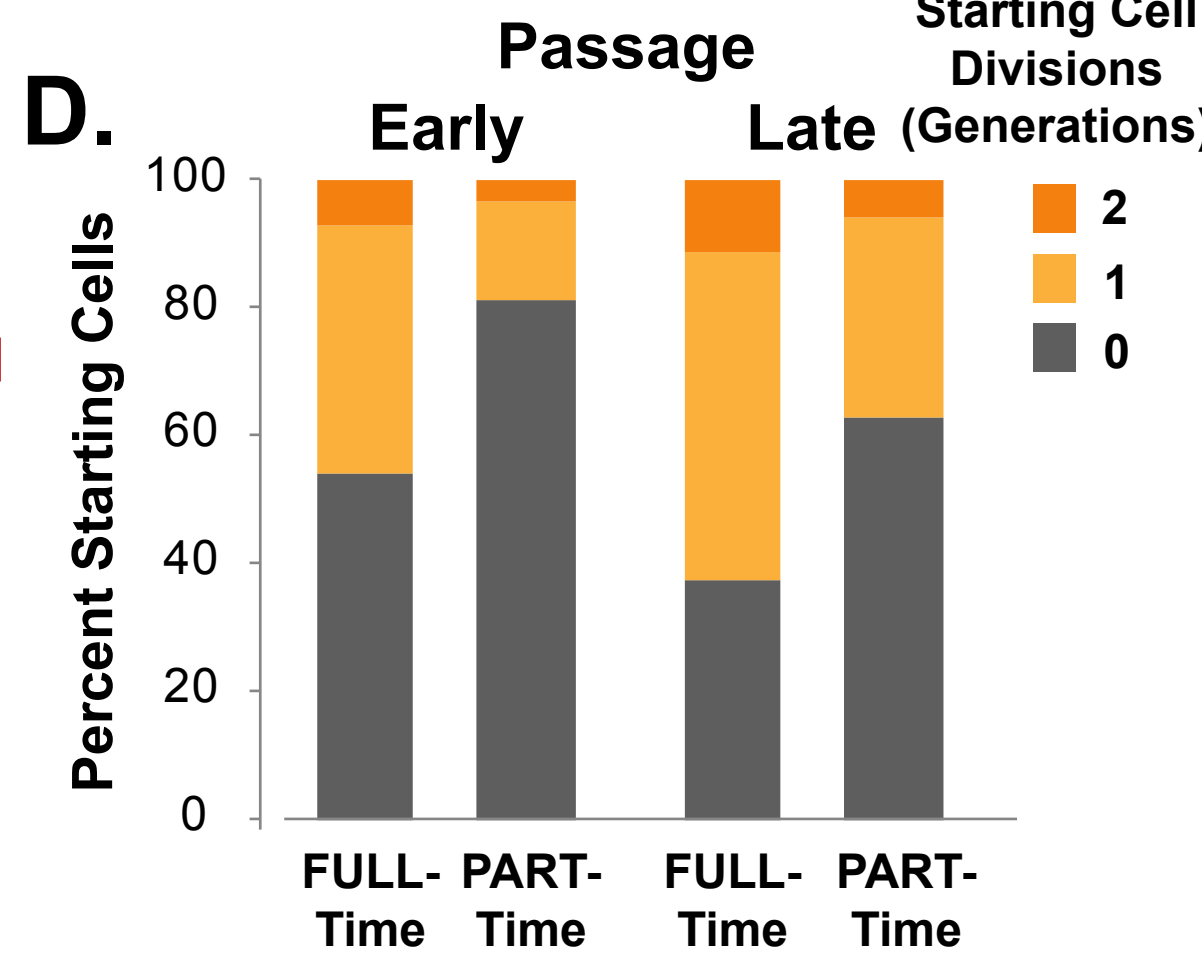


Figure 2. No consistent delay for mitosis. No decrease in total generations. Cell Yields in Cultures Exposed to Room Air are Diminished by Fewer Cells Dividing at All. (A, B) Frame-by-frame cell fate analysis of two sets of videos showing mitotic events per 15 mins in cells cultured under Part-Time Oxygen Control (cell handling in room air conditions) or Full-Time Control (5% O₂/5%CO₂ for cell incubation and handling). A lag phase of reduced mitotic activity after trypsinization ends in a small burst of mitotic events between 24 and 30 hours of incubation. A few mitotic events occurred while cells were adhering after passage. No consistent delay in the initiation of cell division was evident. (C.) Each line represents an individual cell in this tracing of individual cell lineages. Cycling ells were capable of dividing once or twice in either set of conditions. The cells exposed to room air during cell handling consistently exhibited fewer mitotic events as individuals and as a population. While a small sampling, it suggests that cells are more likely to divide when protected from room air during cell handling.

Conclusion

Full-Time atmospheric control during cell handling increased cell yield by protecting the ability of individual cells to resume the cell cycle after passaging, reducing the numbers of cells in permanent cell cycle arrest.