

# Full-Time Physioxic Cell Culture and Handling Improves MSC Proliferation Over Hypoxic Pre-conditioning *in Vitro*

Program  
#651.8

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## ABSTRACT

Cells cultured and handled in traditional room air experience a dramatic shift in oxygen levels when administered *in vivo*. It has been reported that hypoxic pre-conditioning of mesenchymal stem/stromal cells (MSC) in culture can change cell function, increasing cell survival *in vivo* after administration, changing cell migration and modulating cytokine and exosome secretion patterns. These treatments place cells in low oxygen atmospheres for lengths of time from 15 minutes to 36 hours, sometimes with a period of reoxygenation in room air conditions before administration, risking oxidative stress. Here, we compare hypoxic pre-conditioning to full-time physioxic conditions with the null hypothesis that *in vitro* culture conditions make no difference to MSC proliferation rates after the cells are introduced to physioxic conditions as if administered. MSC cultures were split into two groups: (1) cells cultured under full-time physioxia (5% O<sub>2</sub>), and (2) cells cultured under supraphysioxic room air incubator conditions (18% O<sub>2</sub>) and then pre-conditioned in low oxygen (1% O<sub>2</sub>) conditions for 24 hours before reoxygenation at supraphysioxic room air incubator oxygen (18% O<sub>2</sub>) for 24 hours. The cells were then exposed to venous blood oxygen levels (5% O<sub>2</sub>) as if injected *in vivo*. Using an immersion oxygen probe, we recorded vessel headspace and pericellular medium oxygen levels under each regimen. Using the PHI Holomonitor M4, we recorded holographic time-lapse images of the cells for changes in cell morphology or proliferation rates. In looking at cell culture oxygen change kinetics, we found that sharp changes in atmospheric oxygen levels were followed by much slower oxygen changes in the vessel headspace, and pericellular oxygen. MSC were actually at low oxygen conditions for less time than the pre-conditioning period. Hypoxic pre-conditioning after incubation at 18% O<sub>2</sub> increased the numbers of cells that failed to divide at physioxic oxygen levels, disproving the null hypothesis. We concluded that full-time physioxic conditions for cell handling as well as incubation may be more conducive to MSC proliferation than hypoxic pre-conditioning.

## BACKGROUND

- Hypoxic pre-conditioning *in vivo* has been of therapeutic interest since the 1970s<sup>1</sup>
- Exposing MSC cultures that have been grown in room air conditions *in vitro* to low oxygen before infusion has been shown to benefit MSC function in culture and after engraftment<sup>2</sup>
- Low oxygen pre-treatment induces HIF-1 $\alpha$  stabilization and downstream gene activation<sup>3</sup>
- We have previously shown that sharp changes in culture oxygen is highly stressful for human bone marrow MSC and can negatively affect cell proliferation rates<sup>4</sup>

## OBJECTIVES

Expose human bone marrow MSC to different oxygen condition regimens *in vitro*:

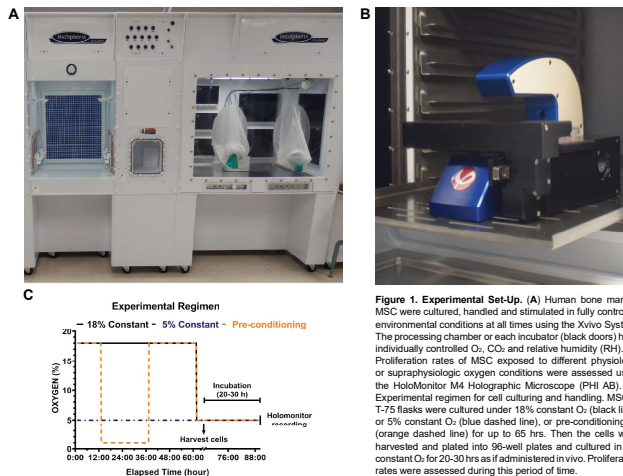
- Constant 18% oxygen (typical room air incubator conditions)
- Constant 5% oxygen (physiologic oxygen in venous blood)
- 18% oxygen with a 1% hypoxic pre-conditioning regimen before return to 18% and then 5% as if cells were harvested in room air conditions and administered *in vivo*

Monitor MSC population growth rates

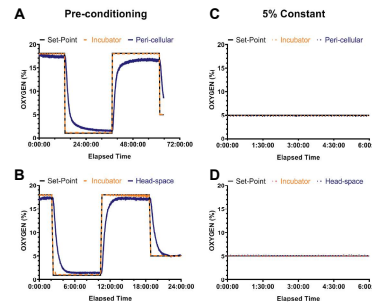
## REFERENCES

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3. Lambertini E, Penoldazzi L, Angelozzi M, Bergamin LS, Manfredini C, Vieceli Dalla Sega F, Paoletti F, Lisignoli G, Piva R. Hypoxia Preconditioning of Human MSCs: a Direct Evidence of HIF-1 $\alpha$  and Collagen Type XV Correlation. *Cellular Physiology and Biochemistry* 2018, 51(5):2237-2249.
4. Henn A, Farrell G, Darou S, Yerden R. Shock from Exposure to Room Air Conditions Alters Individual Mesenchymal Stem Cell Fate, Population Dynamics, and Batch Yields. *International Society of Cell Therapy North America* 2017.

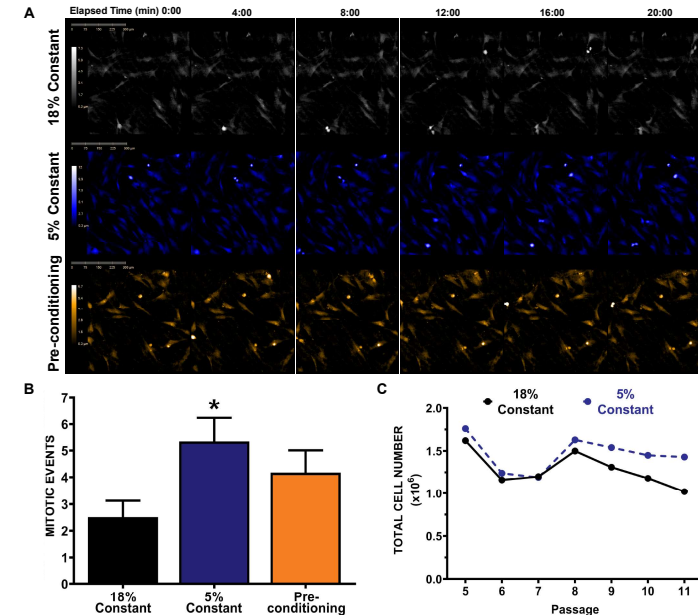
## EXPERIMENTAL DESIGN



## RESULTS



## RESULTS



**Figure 3. Constant Physiologic Oxygen is Better for MSC Proliferation than Hypoxic Pre-conditioning because Initial Room Air Incubator Conditions are Hard on MSC.** MSC in T-75 flasks were cultured in different oxygen controls up to 65 hrs as in fig 2. Then, cells from different oxygen controls were harvested separately, plated into one 96-well plate, and cultured in 5% constant oxygen for 20-30 hrs as if administered *in vivo*. Mitotic events were monitored and recorded using Holomonitor M4 Holographic microscope. Three identical positions in each well were recorded for 3 hrs with 4 min intervals. Images from each position were analyzed and total proliferation events were calculated. (A) Representative images from each group illustrating mitotic events. (B) Data was expressed as mean of mitotic events per well + SEM (n=12). An asterisk (\*) indicates values significantly different from 18% constant oxygen group as determined by ordinary one-way ANOVA followed by Tukey's multiple comparisons test (p<0.05). (C) Total cell numbers of MSC cultured under either 18% constant oxygen or 5% constant oxygen from each passage were graphed. Cell counts paralleled each other until later cell passages when constant 5% oxygen kept MSC growing better in later cell passages. Hypoxic pre-conditioning may increase proliferation after cells are exposed to supraphysioxic room air conditions, but constant physiologic conditions are better.

## CONCLUSIONS

- Cells are not exposed to oxygen changes at the pericellular level that are indicated by chamber oxygen levels
- MSC exposed to hypoxic pre-conditioning are initially exposed to room air incubator conditions which are unfavorable for MSC growth when compared with full-time physioxic conditions
- Cells under physioxic conditions grow for more passages before senescence