FULL-TIME PHYSIOXIC CULTURE CONDITIONS PROMOTE MSC PROLIFERATION AT PHYSIOXIC CONDITIONS BETTER THAN HYPOXIC PRE-CONDITIONING

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ABSTRACT

Cells that are cultured and handled in traditional hyperoxic room air experience a dramatic shift in O_2 levels when injected in vivo. It has been reported that hypoxic pre-conditioning of mesenchymal stromal cells (MSC) in culture can increase cell survival in vivo after administration. Hypoxic pre-treatments subject the cells to lower O_2 atmospheric conditions for lengths of time that range from 15 minutes to 36 hours, with another period of high oxygenation in room air conditions before administration. We previously showed that Human bone marrow MSC yields are improved by enclosing cell-handling processes as well as incubation for a full-time controlled physioxic atmosphere. Here, we used the Xvivo System (BioSpherix), with fully-enclosed and environmentally-controlled cell incubation and handling chambers, to compare different hypoxic pre-conditioning regimens to full-time physioxic conditions. Our null hypothesis was that the different culture conditions make no difference to MSC proliferation rates. MSC were incubated for 12-36 hours at 1% O_2 before return to traditional supraphysioxic room air incubator oxygen levels for 24 hours. The cells were then incubated at 5% O_2 (venous levels) as if injected in vivo. These conditions were compared with unbroken 5% O_2 for both cell handling and culture. Using an immersion O_2 probe, we recorded vessel headspace and pericellular medium O_2 levels during each regimen. We looked for changes in cell division rates. MSC pericellular O_2 levels lagged far behind atmospheric O_2 level changes, so MSC experienced low O_2 conditions for far less time than the pre-conditioning period. Unbroken O_2 conditions were more favorable for keeping MSC in the cell cycle than any pre-conditioning regiment, disproving the null hypothesis. We concluded that hypoxic pre-conditioning is not as favorable for MSC yields as full-time physioxic conditions for cell handling and incubation.

BACKGROUND

- **Exposing MSC cultures grown in room air to low O_2 has been shown to benefit MSC function[1]**
- **Low O_2 induces HIF-1a stabilization and activation of downstream genes involved in cell proliferation[2]**
- **We previously showed that human bone marrow MSC proliferate for more passages at full-time physioxic conditions than in room air culture[3]**

OBJECTIVES

- **Assay MSC proliferation rates after different times at 1% O_2 for hypoxic preconditioning and compare with full-time physioxia (5% O_2)**
- **Assay final cell numbers after treatment**
- **Assess pericellular oxygen levels at each condition**

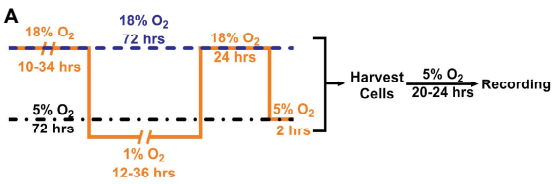
REFERENCES

1. Bader AM, Klose K, Bieback K, Korinth D, Schneider M, Seifert M, Choi Y-H, Kurtz A, Falk V, Stamm C: Hypoxic preconditioning increases survival and pro-angiogenic capacity of human cord blood mesenchymal stromal cells in vitro. PloS one 2015, 10(9):e0138477.

2. Lambertini E, Penolazzi L, Angelozzi M, Bergamin LS, Manferdini C, Vieceli Dalla Sega F, Paolella F, Lisignoli G, Piva R: Hypoxia Preconditioning of Human MSCs: a Direct Evidence of HIF-1α and Collagen Type XV Correlation. Cellular Physiology and Biochemistry 2018, 51(5):2237-2249.

3. 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



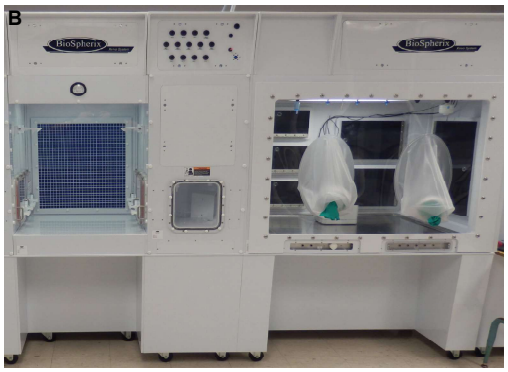


Figure 1. Experimental Set-Up. (**A**) Schematic, dynamic O_2 control for cell culturing and handling. Human bone marrow MSC in T-25 flasks were cultured under 5% constant O_2 (physioxia; black line), 18% constant O_2 (blue line), or 12, 24 or 36 hr of 1% O_2 (hypoxic preconditioning; orange solid line). The cells were harvested and plated into a 96-well plate and cultured at 5% constant O_2 as if injected *in vivo*. (**B**) MSC were cultured, handled and observed in fully controlled conditions at all times using the Xvivo System. Each process chamber or incubator (black doors) had individually controlled temperature, O_2, CO_2 and relative humidity (RH).

RESULTS

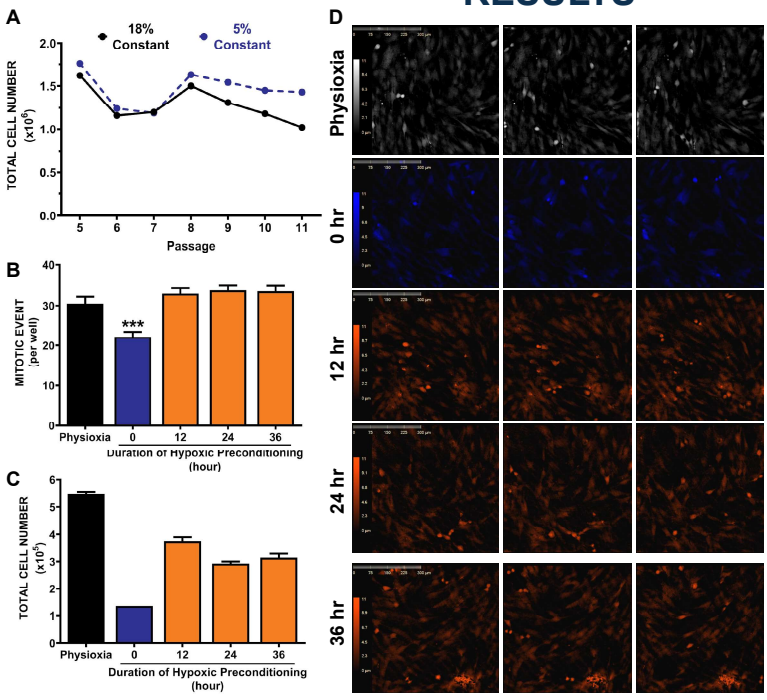


Figure 2. Hypoxic Pre-Conditioning Improves Proliferation over Room Air Conditions, but Not Over Physioxia. MSC in T-25 flasks were cultured in 5% CO_2 plus different O_2 conditions as shown in fig. 1a. Mitotic events were recorded using HoloMonitor M4 Holographic microscope. Three positions in each well were observed for 3 hrs at 6.5 min intervals. Images were analyzed for proliferation events as evidenced by cell rounding. (**A**) Previous data of cell counts at each passage showed faster senescence of MCS exposed to room air O_2. (**B**) Cells cultured as in fig. 1 and plated at 6 x 10e5/ml in 96-well plates showed that hypoxic pre-conditioning can recover cell proliferation rates of room air exposed cells, but not more than constant physiologic O_2. Data was expressed as the mean + SEM (n=42-46 from 4 independent experiments). Asterisks (***) indicate values significantly different from physioxia group as determined by one-way ANOVA followed by Tukey's multiple comparisons test (p<0.01). (**C**) Total cell numbers after culture at different O_2 conditions (duplicate counts). Data expressed as the mean + SEM. **(D)** Representative images.

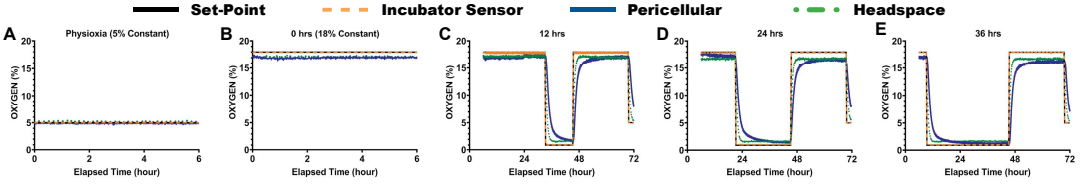


Figure 3. Pericellular and Head-Space Oxygen Levels Lag Behind Incubator Oxygen Changes. Cells were cultured in T-150 flasks. Pericellular and head-space oxygen levels in MSC cultures, and oxygen levels in each incubator were monitored and recorded using an immersion oxygen probe. Cells under physioxic (5% constant oxygen; **A**) and 0 h preconditioning (18% constant oxygen; **B**) were recorded and graphed for 6 hrs. Cells under 12-36 hr preconditioning (**C-E**) were monitored for up tp 72 hrs. Data were graphed from hour 6 to 72. Each figure showed set-point (solid black line) of each incubator, readings from related incubator sensor (orange dashed lines), detected pericellular oxygen levels (solid dark blue lines) and head-space oxygen levels (green dots).

CONCLUSIONS

Pericellular Oxygen Conditions Lag Behind Incubator Atmospheric Oxygen Changes

Hypoxic Preconditioning Increased Cell Proliferation as Compared with Room Air Conditions, but Not as Compared to Full-Time Physioxic Conditions

Overall Cell Growth is Negatively Affected by Room Air Conditions, so Physioxic Conditions are Better