Full-Time Physioxic Control Maximizes Human MSC Expansion at the Individual Cell and Population-wide Levels

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Abstract

Mesenchymal Stem/Stromal Cells (MSC), derived from tissues that normally experience low oxygen levels, are of intense interest for a wide variety of clinical applications including cartilage, skin, and bone repair. Researchers often incubate MSC at physiologic oxygen conditions. However, when handled using conventional room air BSCs, these MSC experience highly variable supraphysiologic conditions and suffer oxidative stress. Using the Xvivo System platform, we can control all temperature and gas levels full-time, during all cell handling steps as well as incubation steps. With robustly controlled conditions, more refined optimization of oxygen levels is possible. Our null hypothesis was that cell growth characteristics of human bone marrow MSC exposed to constant supra-physiologic oxygen conditions would not be different from those exposed to full-time physioxia. Human bone marrow MSC cultures were divided and cultured at 5% CO_2 and 1%, 3%, 5%, or 18% (standard CO_2 incubator) oxygen. The cell processing chamber atmosphere was set to match the incubation conditions for each culture, so each MSC culture was in constant conditions at all times. All solutions were pre-incubated to the appropriate oxygen levels before use. Standard trypan blue counting was used to estimate cellculture densities at each passage and standard colony-forming assays were used to assess clonogenicity. Higher cumulative cell yields and faster cell growth were seen when cells were incubated and handled at 1-5% O_2. On an individual basis MSC were also more likely to retain clonogenic capacity when incubated at these oxygen levels. At the population levels, the MSC produced more passages before senescence when maintained below 5% O_2. This was not an obvious cyto-toxic effect, but an effect upon the number of cells in each generation that remain in the cell cycle. We concluded that constant control of oxygen levels below 5%O_2 can help extend MSC growth beyond that obtained in room air.

Background

- We previously showed that unbroken 5% O_2 produced higher human BM-MSC cell yields than room air O2 at the population-wide and increased the likelihood that individual cells would remain in the cell cycle[1, 2]
- Signalling involving p53 has been implicated[3]
- Room air culture produces higher cellular intracellular ROS and higher stress
- Room air incubators range between 16 and 19% O2, depending upon how often the door is opened

Objectives

- Grown human BM-MSC under constant O_2conditions (both incubation and handling)
- Assess cell yields and clonogenicity at 1%, 3%, 5%, and room air incubator range (18%) oxygen

Experimental Design

1.

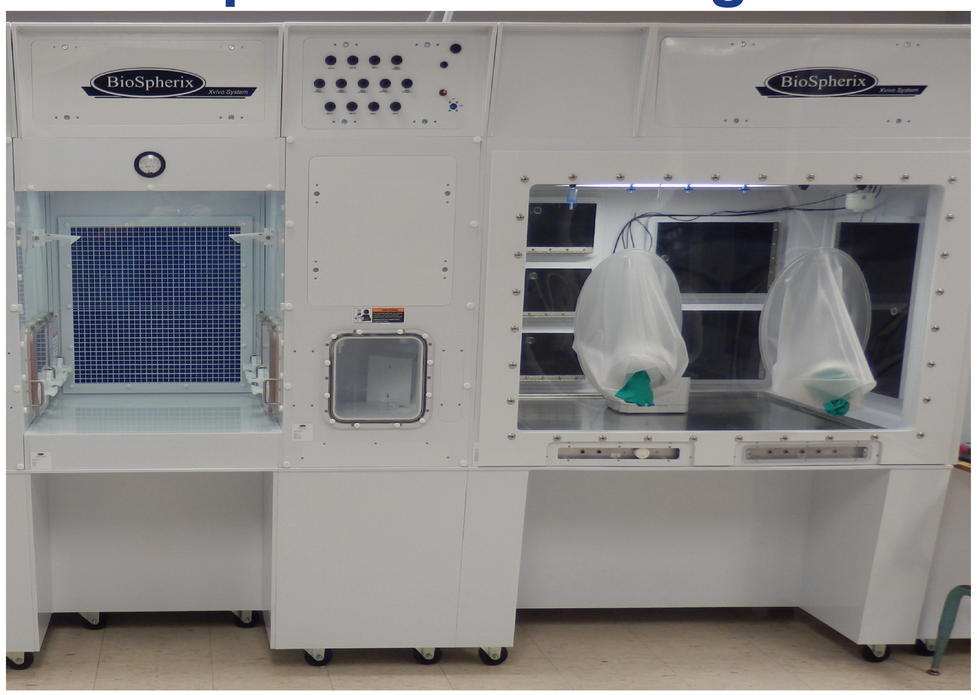

Figure 1. Experimental Design.
Human bone marrow MSC (Lonza) were cultured and handled under completely controlled conditions in an Xvivo System with six indepen-dently controlled and monitored incubators opening into a controlled cell handling space (Process Chamber). CO_2 was controlled to 5% for all processes. O_2 was controlled to 1, 3, 5, or 18% in the incubators as indicated. For cell handling, the processing chamber was set to match the incubator of each culture set. Duplicate T75 cultures were established at each set of conditions with routine trypsinization for passage twice weekly. MSC medium plus singlequot additives and trypsin/ EDTA from Lonza were used.

Results

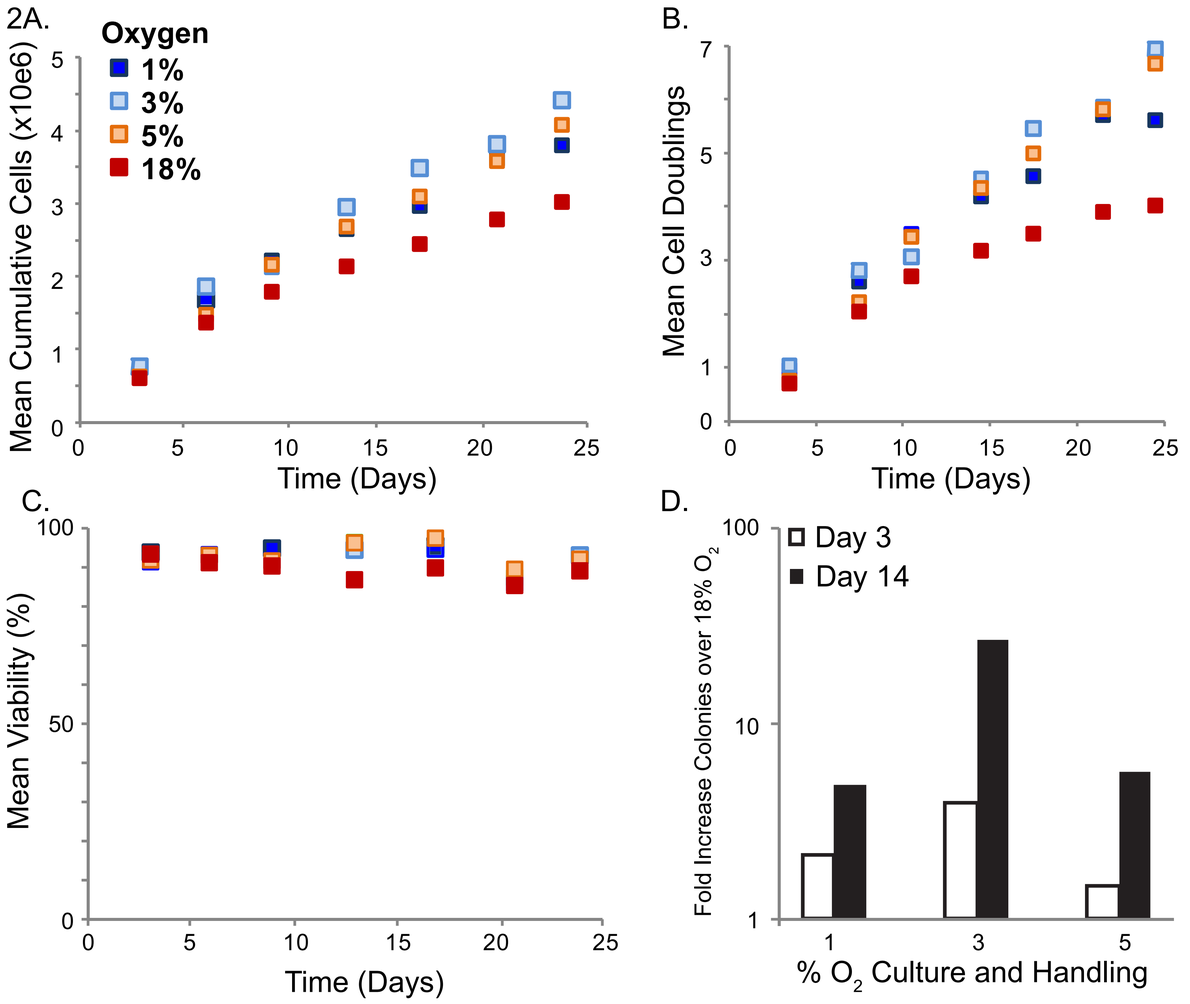

Figure 2. BM-MSC Grew Best at O2 Levels at a Constant 5% or Lower.
MSC grown in unbroken conditions at 5%CO_2 3% O_2 gave slightly higher yields than MSC grown at 1% or 5% as assessed by cumulative cell number (A), and mean cell doublings (B) (formula = t*(LOG(q2/q1)) MSC grown at 18% O_2 grew more slowly than those cultured and handled at constant physioxic range O_2. (C) Viability was 88% or higher for all samples indicating that this was a cytostatic effect rather than a cytotoxic effect. In colony forming assays (D), Cells grown at 3% O_2 formed the most colonies, at both Day 3 and Day14 of cell culture, up to 30-fold higher numbers of colonies than MSC maintained and handled at 18% oxygen. MSC grown at 1 and 5% O_2 also showed more colonies than cells grown in room air range oxygen. Cell numbers and viability were assessed by trypan blue exclusion and microscopic manual counting with a hemacytometer. MSC were plated in soft agar at three 10-fold dilutions and grown for 2-3 weeks before colonies were assessed by microscopic counting. Calculations were done with Excel software (Microsoft).

Conclusions

Room Air Range Oxygen Levels are Detrimental to Human BM MSC Growth
Optimal Constant Oxygen Levels are less than 5% O2

References

1. Henn, A., et al., 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.
2. Henn, A., S. Darou, and R. Yerden, Physiologically Relevant Oxygen during cell Handling as Well as Incubation Enhances the Growth of Human Mesenchymal Stromal Cell Cultures. International Society for Stem Cell Research, 2016.
3. Boregowda, S.V., et al., 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, 2012. 30(5): p. 975-87.
4. Ronn, R.E., et al., Reactive Oxygen Species Impair the Function of CD90+ Hematopoietic Progenitors Generated from Human Pluripotent Stem Cells. Stem Cells, 2017. 35(1): p. 197-206.