

Physiologic Control of Cell Handling Conditions Reduces Variability for Human MSC

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Abstract

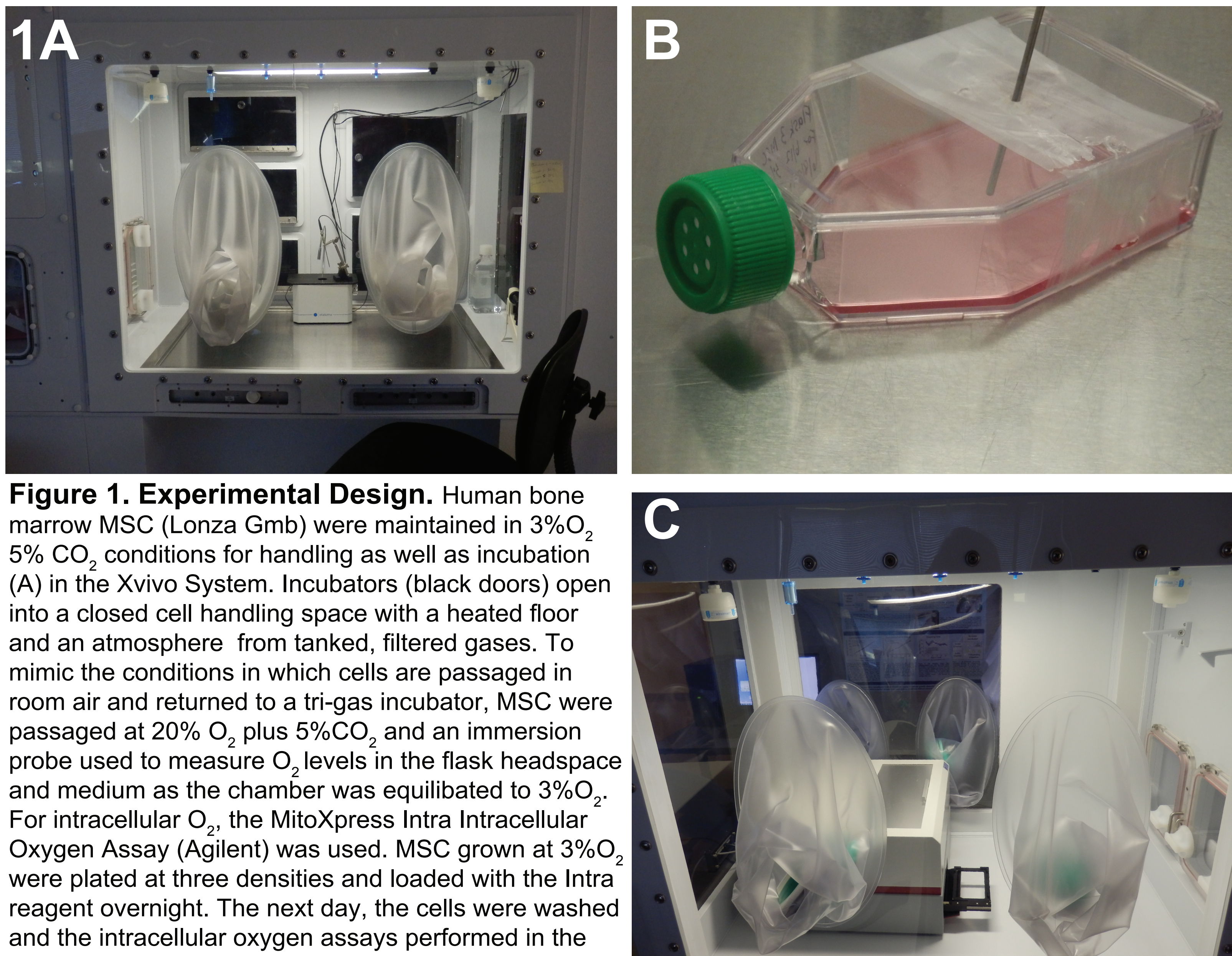
The importance of incubating clinically valuable mesenchymal stem/stromal cells (MSC) at low oxygen levels has been well established, but many cell culturists still handle MSC in conventional room air biological safety cabinets (BSC). This practice causes stress in MSC as they experience swings in conditions and increasing experimental variability. MSC utilize aerobic glycolysis which consumes less O_2 than oxphos even when O_2 is abundant. We have previously published that room air cell handling reduces MSC yields and speeds culture senescence. For this project, we sought to establish how long it takes pericellular O_2 to return to optimum low levels after MSC undergo routine passaging in a room air atmosphere. Using the Xvivo System, in which we control gas levels and temperature for the cells during all cell handling steps as well as incubation, we sealed an immersion oxygen probe into a vented-cap T-75 flask of human bone marrow MSC cells immediately after passage. We kept the flask in the cell handling chamber, which was at $37^\circ C$ and 20% O_2 , and set the atmosphere to 3% O_2 . We monitored pericellular O_2 levels as the chamber atmosphere, the vessel headspace, and the cell culture medium equilibrated to 3% O_2 . With 15ml of medium sitting undisturbed, we found that it took between 1 and 2 hours for pericellular O_2 levels to equilibrate to chamber oxygen levels. This is a long period of time for MSC to be in stress-inducing supra-physiologic oxygen levels. When cell culture medium was pre-equilibrated and conditions controlled, the cells stayed at optimum levels. We concluded that full-time control of cell handling atmosphere is necessary to reduce variability in conditions for MSC.

Background

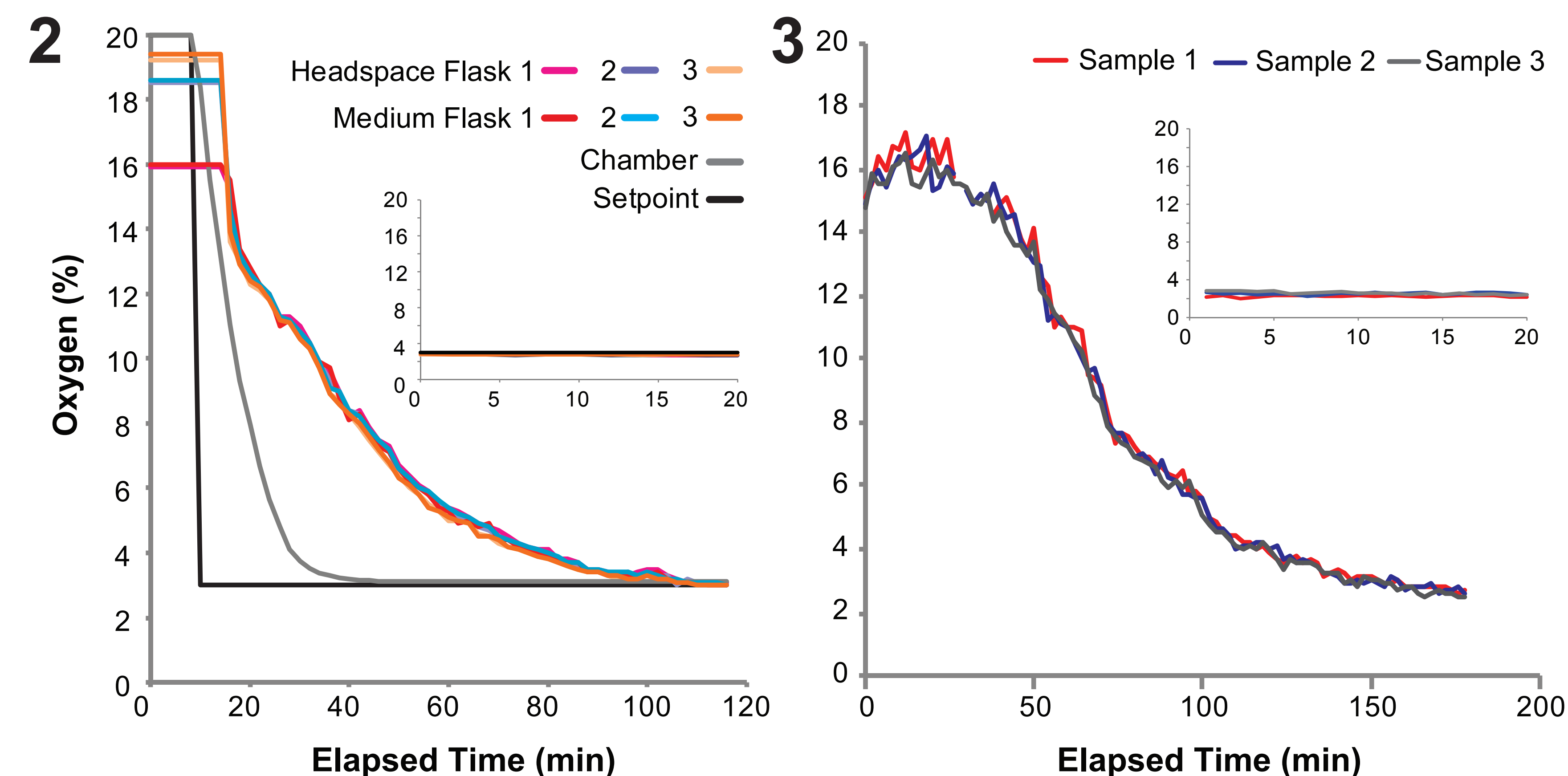
Room air oxygen¹ and changes in oxygen levels² are stressful to MSC and can affect MSC function³. Human MSC tend to use aerobic glycolysis³, and are negatively impacted by increased ROS in room air culture¹.

Null Hypothesis: Cells are not out of optimum for more than the 5 min it takes to change medium in a room air BSC and return them to a tri-gas incubator

Experimental Design



Results



Conclusions

Hypothesis disproved. Cell handling in room air O_2 produces out-of-optimum pericellular and intracellular conditions for hours after cells are back in the incubator.

Cell handling in full-time controlled conditions prevents these out-of-optimum variations in conditions.

References

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