

ADAPTATION TO SUPRAPHYSIOLOGIC ROOM AIR OXYGEN CHANGES THE EFFECTS OF NITRIC OXIDE ON A549 LUNG CELLS IN VITRO

Alicia D. Henn, Shannon Darou, Randy Yerden. BioSpherix, Ltd. Parish, NY

Abstract

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Airway epithelial cells are exposed to some of the highest O₂ levels encountered *in vivo*. However, O₂ levels, even in the lungs, are far lower than room air (~10% vs 21% O₂, respectively). The culture and handling of airway cells in room air can alter the redox status of the cells, introducing artifact. Nitric oxide (NO), which can affect HIF-1alpha stabilization and bind cytochrome oxidase, has been shown to play multiple roles in the response of lung to hypoxic injury and inflammation. Here we explore whether pre-adaptation of the commonly used human lung epithelial cell line A549 to room air O₂ levels changes cellular responsiveness to NO. Using the Xvivo System to provide full-time control of O₂ and CO₂ during cell incubation as well as handling, we adapted A549 cells to 18% supraphysiologic O₂ (standard room air incubator with humidity and CO₂), physiologic O₂ (10%) or pathologic hypoxia (2% O₂). We assayed cellular responses to exposure to NO in standard plate-based cytotoxicity assays. We found that adaptation to different O₂ levels had a concentration-dependent effect on the responses of A549 cells to NO. We concluded that it is critical to consider the O₂-adapted state of the cell prior to assay to avoid O₂ artifact.

Background

- Room air incubators have much higher O₂ levels than tissues *in vivo*¹
- Culture in room air can alter cells' redox capacity, introducing artifact²
- The O₂ history of the cell can change NO production and affect cell responses to NO³
- Inhaled NO can affect lung development⁴

Objectives

- Thoroughly adapt A549 human lung carcinoma cells to 2%, 10%, and 18% (standard room air incubator) O₂
- Assess if responses to gaseous NO are changed

References

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Experimental Design

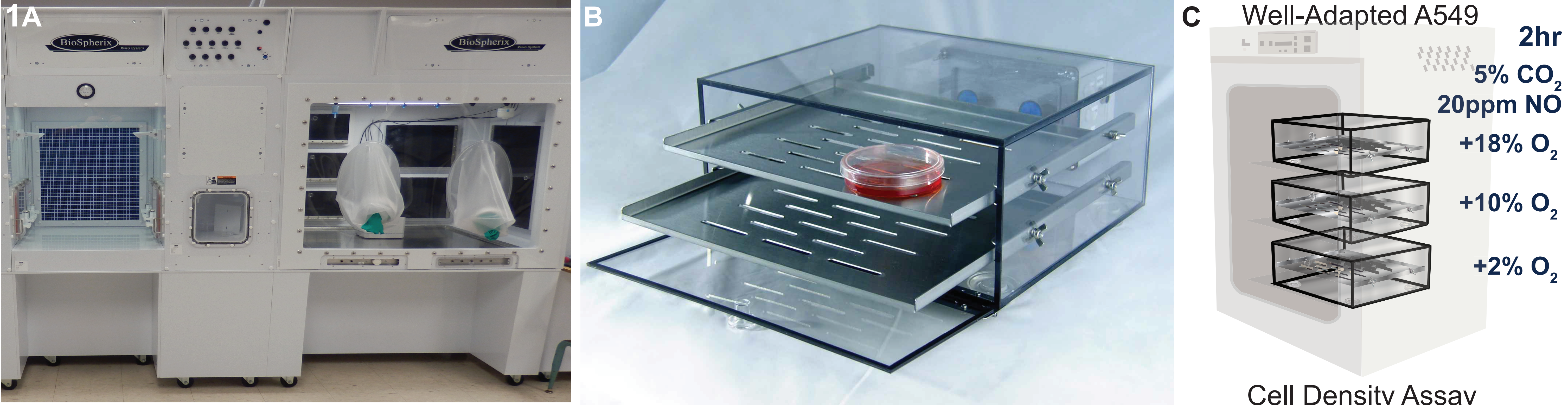


Figure 1. Experimental Design. (A) The Xvivo System was used to maintain A549 cells in constant conditions during cell handling as well as incubation. Incubators (black doors) opened only into a sealed cell handling space supplied with tanked, medical-grade gases. The processing chamber floor and air were heated and the air was continuously HEPA filtered. RPMI 1640 + 10% FBS + L-Glutamine (Gibco) with no antibiotics was pre-equilibrated to the proper O₂ level for at least 12 hr before use. A549 were plated (18 wells/sample/plate with the rest of the wells filled with PBS) in 96-well plates at 2500 cells/well O/N before 2hr exposure to 20ppm NO for in a standard CO₂ incubator subchamber (B) equipped with an OxyCycler GT4181N controller. (C) Schematic. Cells were returned to the Xvivo incubated 24, 48, or 72 hr before assessment for cell density using a standard crystal violet assay.

Results

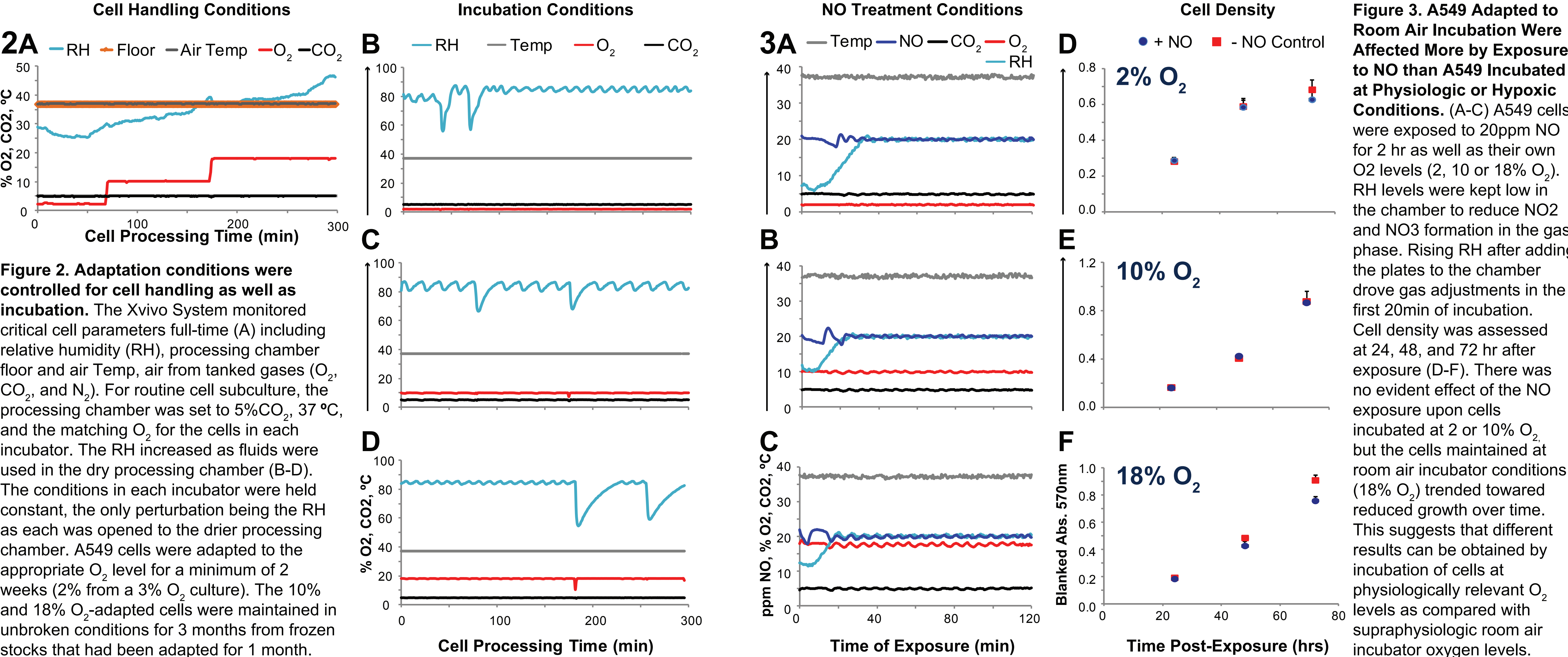


Figure 2. Adaptation conditions were controlled for cell handling as well as incubation. The Xvivo System monitored critical cell parameters full-time (A) including relative humidity (RH), processing chamber floor and air Temp, air from tanked gases (O₂, CO₂, and N₂). For routine cell subculture, the processing chamber was set to 5%CO₂, 37 °C, and the matching O₂ for the cells in each incubator. The RH increased as fluids were used in the dry processing chamber (B-D). The conditions in each incubator were held constant, the only perturbation being the RH as each was opened to the drier processing chamber. A549 cells were adapted to the appropriate O₂ level for a minimum of 2 weeks (2% from a 3% O₂ culture). The 10% and 18% O₂-adapted cells were maintained in unbroken conditions for 3 months from frozen stocks that had been adapted for 1 month.

Figure 3. A549 Adapted to Room Air Incubation Were Affected More by Exposure to NO than A549 Incubated at Physiologic or Hypoxic Conditions. (A-C) A549 cells were exposed to 20ppm NO for 2 hr as well as their own O₂ levels (2, 10 or 18% O₂). RH levels were kept low in the chamber to reduce NO₂ and NO₃ formation in the gas phase. Rising RH after adding the plates to the chamber drove gas adjustments in the first 20min of incubation. Cell density was assessed at 24, 48, and 72 hr after exposure (D-F). There was no evident effect of the NO exposure upon cells incubated at 2 or 10% O₂ but the cells maintained at room air incubator conditions (18% O₂) trended toward reduced growth over time. This suggests that different results can be obtained by incubation of cells at physiologically relevant O₂ levels as compared with supraphysiologic room air incubator oxygen levels.

Conclusions

Adaptation to room air oxygen can alter the responses of human lung cells to nitric oxide. Maintain cells at unbroken physiologic oxygen for more physiologically relevant results.