From tissue physoxia to cancer hypoxia, cost-effective methods to study tissue-specific O2 levels in cellular biology

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INTRODUCTION

• Oxygen concentrations play distinctive biological roles in cellular metabolism, and low concentrations may be crucial to natural homoeostasis.

• Interestingly, physiological concentrations of oxygen (1% - 11%) are closer to hypoxia (<2%) than to the atmospheric oxygen concentration (~21%), which has implications for most investigations using cell culture nowadays, considering that the atmospheric concentration is widely misapplied to reflect physoxia.

• Current methods to recapitulate in vitro physoxia or hypoxic conditions are limited and range from lower-cost chemical-induction of hypoxia-like effects with cobalt chloride to costly equipment with nitrogen-injection chambers.

OBJECTIVE

• We present a cost-effective alternative to culture cells at hypoxic and physoxic conditions by controlling the precise concentration of oxygen with an oxygen absorber containing active powdered iron oxide.

• This deoxidizing absorber method uses an oxygen absorber, cells, and an oxygen meter inside a double-sealed bag.

• The objective of this study was to investigate if utilization of this technique can reproduce a typical cell response to hypoxia, including acquisition of an EMT phenotype, increased levels of reactive oxygen species (ROS) and superoxide dismutase (SOD), cell cycle arrest, and the activation of tumorigenic pathways observed in hypoxic tumors, including activation of the mTOR pathway.

METHODS & RESULTS

DISCUSSION

• Deoxidizing absorbers as a method for hypoxia enable researchers to modulate oxygen concentration to more accurately reflect hypoxia, physoxia, or normoxia.

• Notably, the use of this technique facilitates acquisition of acute and chronic hypoxia, which are difficult to achieve with current methodologies, in addition to being a cost-effective method.

• Validity of this technique was demonstrated through its ability to replicate routine hypoxic events, including expression of EMT phenotype, increased levels of ROS, activation of the mTOR pathway, as evidenced through pS6 expression, as well as induction of cell cycle arrest and CSC accumulation.

CONCLUSION

• The deoxidizing absorbers method was successfully able to reproduce acute and chronic forms of hypoxia and physoxia, thereby providing a reasonable, cost-effective alternative to current methodologies.

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