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BAKER SCIENTIFIC APPLICATION BULLETIN

# Physoxia is the basis of good cell culture practice

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## CONTENT

The result of any experiment conducted in a life science laboratory is only as good as the material used in the experiments. Whether working with cells, tissues or any other material one needs to pay attention to all the variables the samples will be exposed to throughout the experimentation.

Cells are highly sensitive to disturbances in their environment. They may not necessarily react through radical responses like apoptosis, but a number of subtle, long term effects take place in cells in response to variations in temperature, pH, confluency, light, media composition etc.

It is difficult to fully optimize in vitro conditions and to take everything into account, but in order to do the best science possible it is important to take all the possible measures to ensure high quality experimental results.

Oxygen is the key molecule to life. As the electron acceptor in mitochondrial oxidative phosphorylation it drives cellular processes crucial to all cellular events from metabolism to signalling to redox homeostasis. However, it is not the mere presence of oxygen that is important to most life forms but it is the level of O2 that is the key element.

Cellular functions occur optimally in a quite narrow oxygen range; the lung epithelial cells reside in approximately 10-14% O2 whereas brain cells function in O2 levels of 2-4%. This is to say, exposing cells to anything above or below their optimal, natural O2 environment will have consequences that are difficult or impossible to track let alone understood. Introducing such strong disturbances as non-physiological oxygen levels will leave any result obtained from such an experiment open to interpretation.
On systemic level oxygen sensing occurs through chemoreceptor cells. They function by regulating cardiovascular and ventilatory rates. When oxygen availability is compromised a cascade of chemosensory systems modulate blood circulation, pulmonary ventilation and perfusion in order to optimize oxygen delivery to cells and tissues. These responses base on the function of carotic bodies in the arterial circulation as well as neuroepithelial bodies in the airway.

One cell type that responds directly to low oxygen conditions are the vascular smooth muscle cells. They are responsible of the rapid response vasoconstriction of pulmonary arteries. This response is initiated by the inhibition of membrane potential setting potassium channels and in turn activates voltage-gated calcium channels, followed by elevated cytosolic calcium levels causing myocyte constriction (Post et al).

Hypoxia also induces vasodilation occurring especially in coronary and cerebral vessels. This is partly mediated by vascular smooth muscle KATP -channels which open up when ATP production is decreased in response to hypoxia (Dart et al).

More acute cellular responses occur through the activation of oxygen sensitive proteins and ¬through gene transcription regulation. These hypoxic responses work to increase the effectiveness of the energy-producing pathways (increased anaerobic glycolysis) and at the same time to decrease energy-consuming processes. Most of the energy used goes to synthesizing proteins and running the ion-motive ATPases. Under hypoxia ATP supply is lowered and thus it is important for cells to allocate cellular energy wisely to essential and non-essential ATP-demanding processes. Hence protein and DNA/RNA synthesis are the first to be shut down in hypoxic conditions and Na/K -pumping and Ca2 cycling being given priority (Boutilier RG).

When cells face the opposite condition, namely hyperoxia (in cell culture 21% O2 constitutes as hyperoxia), similarly radical events take place. Levels of reactive oxygen species (ROS) are increased provoking DNA damage, lipid peroxidation and activation of genes that increase inflammation and cell death (Kwak et al)

The above examples of cellular reactions to varied oxygen concentration highlights the sensitivity and flexibility of these systems. At the same time it indicates that introducing cells with stressors like hyperoxic conditions in regular CO2 incubator will push cells to react in a non-physiological way. These responses can be very profound and in worst case scenario irreversible. Cellular sensitivity must be well understood in order to distinguish true responses to experimental setups and what is only consequence of unsuitable cell culture conditions.

## REFERENCES

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