Redefining "Biologically Relevant" in Relation to Oxygen Availability

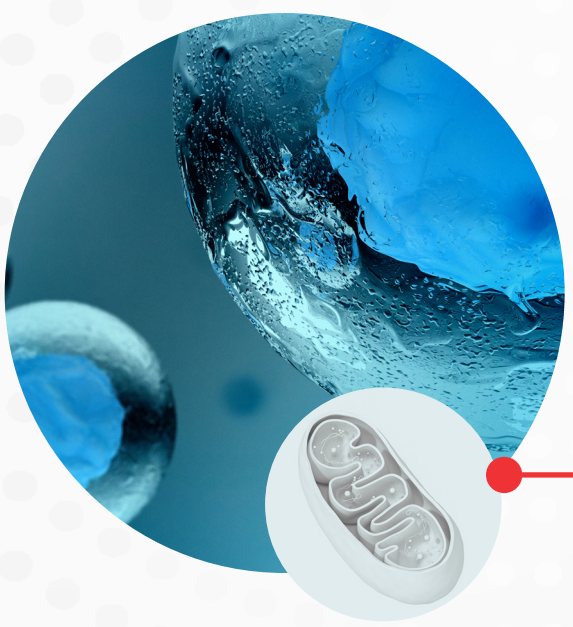
Cell culture is a vital part of biological research. However, cell culture is not always conducted in a manner that preserves physiological relevance and optimizes cellular health. In particular, oxygen availability during in vitro culture is often excessive, causing cells to behave abnormally, thereby decreasing data consistency and workflow efficiency.

What is Physiological O_2?

Scientists commonly culture eukaryotic cells in atmospheric conditions containing 20-21% oxygen. This value mimics the oxygen content of the Earth's atmosphere and has become the reference point for the term "normoxia."[1] However, owing to diffusion properties and differences in pressure, oxygen concentrations available to cells inside the body ("physioxia") are typically much lower. Moreover, available oxygen abundance varies dramatically from tissue to tissue, ranging from 14% in the lungs to ~1% in parts of the brain, bone marrow, and eyes. This discrepancy has tremendous implications when it comes to designing in vitro models to mimic in vivo conditions.

Oxygen Levels in Human Tissues

Lungs	14%
Arteries	12%
Liver, heart, kidneys	4-12%
Eyes	1-5%
Brain	0.5-7%
Bone marrow	0-4%



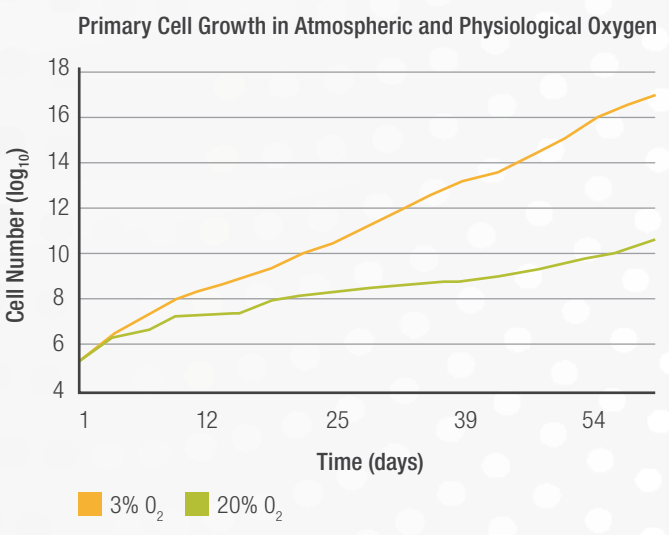
How Does Oxygen Affect Cells?

Oxygen's primary physiological role is to facilitate ATP production in aerobic respiration, particularly via the electron transport chain. The high levels of energy produced by this process are necessary for eukaryotic life.[1] Because oxygen is critically important, cells are very sensitive to its levels and availability. Hypoxia and hyperoxia both affect cellular gene and protein expression, with considerable effects on metabolism, growth, and differentiation. Furthermore, oxygen-mediated behaviors are often threshold-dependent, meaning that small shifts can trigger disproportionate responses.[2] Elevated oxygen levels can translate to elevated production of reactive oxygen species, leading to oxygen toxicity and reduced cellular health and viability.

Physioxia and Hypoxia in the Laboratory

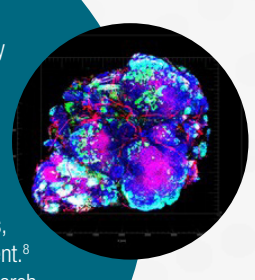
Scientists have demonstrated that physioxic environments are more conducive to cell culture wellbeing than normoxic ones containing 20-21% oxygen. For example, mouse embryonic fibroblasts (MEFs) cultured in a 3% oxygen environment proliferated much more quickly and reached higher saturation densities than counterparts cultured in 20% oxygen.[3] Similarly, human adipose-derived stem cells proliferated more quickly when cultured in 2% oxygen compared to 20%.[4]

Importantly, stem cells cultured under physioxic conditions show reduced oxidative stress, telomere shortening, and chromosomal instability.[5] Furthermore, they live longer while also maintaining stemness and avoiding senescence for extended durations.[3,4] Finally, the benefits of physioxic culture conditions can extend post-differentiation. For example, bone marrow-derived mesenchymal stem cells expanded in 2% oxygen showed improved cartilage repair capabilities when introduced in vivo.[6]



Recapitulating the Tumor Microenvironment

Altered oxygen availability plays a role in many disease mechanisms,[1] ranging from neurological and cardiac ischemia to metabolic disorders.[1,7] Of these, hypoxia's involvement in tumor formation and progression is the most well known. A hallmark of the tumor microenvironment, local hypoxia is a major deleterious factor, impeding immune responses, driving malignant progression, and hindering treatment.[8] To create suitable cellular models for tumor research, researchers must recognize that tumor oxygen availability is considerably lower than even physioxic conditions, ranging from 0.3% to 4.2% depending on location and tissue of origin.[8]



Controlling Oxygen in Cell Culture

Scientists must precisely determine and maintain the amount of oxygen in a given environment when creating research models designed to investigate the effects of hypoxia and physioxia in disease processes. This also maximizes cellular health and stability in culture, which is critical when working with stem and primary cells that are highly sensitive to external environmental perturbations.

Researchers use "tri-gas" incubators to create hypoxic cell culture conditions. These incubators apply external carbon dioxide and nitrogen to regulate oxygen levels, and are generally capable of attaining oxygen levels as low as 1% (lower levels cannot be achieved without a closed system). Sensors specific for each gas also allow researchers to closely monitor the composition balance, which is important because too much or too little CO_2 can alter culture pH.

To maximize the advantages of using tri-gas incubators, researchers must take several things into account.

- **Gas distribution:** Oxygen, nitrogen, and carbon dioxide gases have different densities, which can lead to environmental stratification. For example, nitrogen, as the lightest of the three gases, will be disproportionally present at the top of the incubator. Active air circulation is required to avoid creating region-specific microenvironments. A well-designed, fan-based system ensures a uniform atmosphere throughout the incubator so that cells exhibit consistent responses regardless of their position in the chamber.
- **Condition consistency:** Air circulation plays a large factor in accelerating recovery from environmental disturbances, such as when an incubator door is opened, as proper airflow is integral for temperature and humidity distribution. Furthermore, air circulation coupled with HEPA filtration acts as a constant screening process against environmental microorganisms and particles, limiting their ability to settle and contaminate the cell culture vessels themselves. Internal segmentations within the incubator further reduce environmental variation by allowing researchers to access only small portions of an incubator's total storage area at a time.

Researchers can take steps prior to incubation to ease the transition from atmospheric to incubator conditions, such as using deoxygenated culture media.[10]

- **Environmental monitoring:** Constant monitoring provides vital feedback on whether there are unexpected or undesired conditions, although sensor technology has limitations. For example, oxygen level readings below 1% are not trustworthy. Such low numbers fail to account for the margin of error present in all current sensor technology, as well as the residual oxygen remaining in media and within the culture vessels themselves.[9] Consistently achieving oxygen levels below 1% requires a closed system.

For more information on how oxygen levels during cell culture affect cellular properties, please visit **thermofisher.com/safercells**

References

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