Advantages of Oxygen Control in CO_2 Incubation for Research and Clinical Applications

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Physiological in vivo oxygen concentrations can range from 1% to13%, yet most research and culture is done at ambient 21% O_2. Studies have shown dramatic differences in gene expression profiles and phenotypic changes in the cells between these conditions. Scientists in a variety of emerging fields are coming to understand the value of O_2 control in addition to CO_2 and temperature control in their daily applications. New Brunswick Scientific's (NBS's) CO_2 Incubators are offered with three levels of oxygen control. The most popular is the 1–19% option for hypoxic studies. For more stringent requirements, there is a 0.1–19% option. Lastly, for a more robust application dynamic, NBS offers a 1–95% control for both hypoxic and hyperoxic tri-gas control (CO_2, N_2, and O_2).

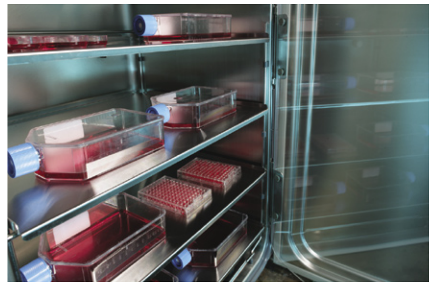

Figure 1. New Brunswick Scientific's fanless, convection-heated CO_2 incubators are uniquely designed to meet the diverse needs of today's lab. A variety of sizes, models and options are offered, including models capable of maintaining hypoxic and hyperoxic conditions for applications ranging from in vitro fertilization (IVF), to mammalian cell culture, to growth of anaerobic bacteria.

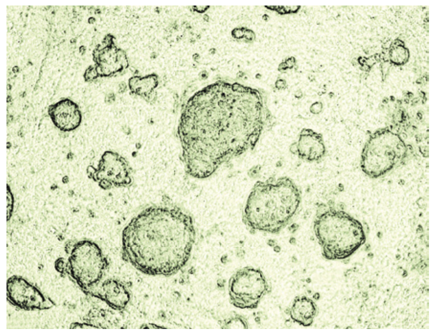

Figure 2. Embryonic stem cells cultured at lower oxygen levels have shown an increase in clonogenicity.

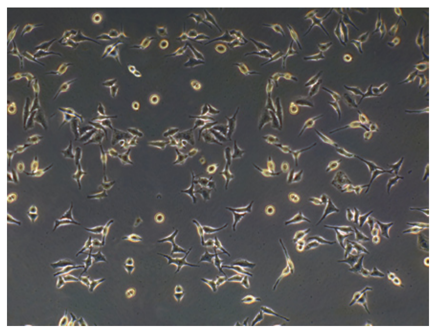

Figure 3. HELA cervical cancer cells.

Applications

Stem Cell Culture

The untapped clinical value in the pluripotency of human embryonic stem cells (hESCs) will yield countless new therapies and potential drug targets in the coming years. Recent studies have demonstrated that culturing hESCs in 2% O_2 (physiologic normoxia) results in smaller cells with reduced complexity, reduced spontaneous differentiation, increased clonogenicity, reduced spontaneous chromosomal aberration frequencies, and extensive propagation of specialized clonal derivative cells compared to culturing in ambient O_2 concentrations. In addition, it has been found that the O_2 concentration necessary for optimal growth and study varies dramatically among species and cell types.

Oncology

As a tumor progresses and expands, it quickly outgrows the blood supply leaving the interior portions of the tumor with much lower oxygen conditions than normal cells. These cells are traditionally more resistant to common cancer therapies due to the slower growth and the limited vascularization. By reducing the environmental O_2, the researcher can mimic these intratumor conditions in the hopes of finding more penetrable therapies.

The transcription factor, complex hypoxia-inducible factor (HIF-1) controls the expression of most genes involved in adaptation to hypoxic conditions. The oxygen-dependent regulation of HIF-1 is a multistep process that includes degradation under normoxia, but stabilization, translocation into the nucleus, and activation, under hypoxic conditions. Hypoxia is associated with angiogenesis and tumor progression. It activates a signal cascade that leads to the stabilization of HIF-1 and activation of genes in the hypoxia cascade. HIF-1 is over-expressed in the majority of human cancers, and its over-expression correlates with poor prognosis and treatment failure.

Toxicity

Clinical drug development is an increasingly costly endeavor. The scientific community has been searching for more predictive models and assays that will allow drug candidates to be evaluated for potency, toxicity, and efficacy prior to entering expensive animal trials and human trials. Due to the gene expression differentials between normoxic and hypoxic conditions in cell lines, the drug interaction, toxicity, and resistance profiles can also vary dramatically between the two conditions. This can result in false results when screening and cause considerable delays and costs when these toxicities are found downstream. By testing at physiological O_2, the cells will more closely mimic the in vivo environment which the compound will present itself in, and behavior will be more predictable to in vivo results.

Microbiology

The recent rise of clinically relevant strains of *Clostridium difficile* has refocused the new antimicrobial development bull's eye onto this emerging nosocomial pathogen. *C. difficile* is an obligate anaerobe that currently requires gas jars in order to obtain optimal growth. Gas jars provide a suitable environment, but are lacking in temperature and gas control. In addition, the capacity inside of each jar is minimal, preventing high throughput applications from being run consistently.

Summary

As cell culture progresses, the standard CO_2 incubator is no longer able to meet the growing demands of modern research and clinical applications. As the understanding of cell biology has become more extensive, scientists have realized that simply growing cultures at 5% CO_2 is not adequate to represent an in vivo environment. Factors such as oxygen, humidity, temperature, and CO_2 need to be modulated in order to provide the most optimal environment for the specific application. Therefore, the CO_2 incubators offered by New Brunswick Scientific provide the flexibility as well as the sophistication to allow for precise control.

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