When Ex Vivo Does Not Reflect In Vivo: Why Traditional Cell Culture Methods Lead to Inaccurate Biological Results

Despite their prevalence in biology labs, traditional incubators do not accurately replicate the native microenvironment of the cells they incubate. This leads to disparities between cell profiles in vivo and ex vivo and calls into question many findings based on cultured cells. A new approach to culturing includes settings for oxygen and pressure levels, more accurately recreating the cells’ original environment and producing more biologically relevant results.

The invention of techniques for growing cells ex vivo was a major leap forward in biology, allowing scientists to study accessible living cells for a better understanding of their function, makeup, and activity. The methods and instrumentation for culturing cells have not changed significantly in the six or seven decades since they were first established. Today, labs may choose to buy incubators — from fairly small designs to extremely large versions — or build homebrew incubators, jury-rigged from items like Mason jars. But the underlying technology behind these incubators is remarkably similar to that used for the first successfully established human cell line in 1951.

Cell culturing is a fundamental technology at biology labs in academic and pharma/biotech organizations around the world, and incubators are critical to enabling that process. But recently, in-depth studies of the cell culturing procedure have revealed important limitations in current technologies. Perhaps the most alarming is that because incubators do not accurately represent the native microenvironment of most cells, the results generated from these studies do not reflect the natural biology of cells.
Culturing Conditions Dictate Gene and Protein Expression

Indeed, research has now shown that growing cells in traditional CO\textsubscript{2} incubators dramatically alters their gene and protein expression, ability to divide into new cells, and other important biological factors like metabolic processes. There is increasing awareness among biologists that cells adapt to new environments within a matter of hours. When cells move to a new environment — whether in the body or to a traditional incubator — it alters their behavior. As a consequence, scientists interrogating cells in an incubator that doesn’t mirror their native environment lose the ability to make meaningful insights, since the cells no longer reflect their normal biology.

A new approach to culturing that more closely mimics the cells’ native microenvironments has been shown to produce more physiologically relevant results from the cells it houses. With this technology, scientists may now study cultured cells with greater certainty that the data they generate from them offers a real representation of how those cells function and behave in vivo.
Ex Vivo vs. In Vivo

In the past several years, many new studies have revealed the disparity between results obtained from cells in vivo and those from cells in traditional incubators. Stem cells, for example, need a very specific environmental niche to differentiate into particular cell types; that process has been quite challenging to replicate through typical cell culturing techniques. Tumor cells stored in incubators have been found to have different signaling patterns than those taken directly from a patient. A parallel trend, the frequent inability to reproduce biological results published even in high-quality scientific papers, is now thought to be partially related to inaccurate results based on the altered profiles of cells in storage.

Scientists have known for decades that the microenvironment is essential to a cell’s function. As postulated by Francis Crick, the central dogma of molecular biology posits that “DNA makes RNA and RNA makes protein.” This principle is articulated in the concept that every cell within the body contains the same DNA content, but what makes a brain cell different from a skin cell is variation in RNA and protein content, based at least partly on environmental factors.

Indeed, seminal work from breast cancer researcher Mina Bissell at the Lawrence Berkeley National Laboratory helped demonstrate that cells lose their tissue-specific function when cultured. “However, the cellular identity is not lost permanently, as we have learned that by controlling the microenvironment of the cells in culture, we can make them ‘remember’ many of their original tissue specific traits,” Bissell wrote (Bissell and LaBarge).

Ex vivo studies of cells are only as valuable as the data they produce, and if that data does not reflect true biology, their value is questionable. Scientists have been attempting to determine which conditions might be adjusted to make incubators more closely reflect a cell’s natural world. For instance, scientists at Brown University grew a neuron in three-dimensional conditions, rather than the traditional flat environment of an incubator plate (Li). They found that hundreds of genes changed their expression patterns based on that single difference.

Importance of Pressure and Hypoxia in Cell Culture

Two characteristics that appear particularly important in reproducing a cell’s microenvironment are pressure and hypoxia. Most traditional incubators maintain mammalian cells at 37 degrees Celsius with 5% CO₂ and 85% humidity. This one-size-fits-all approach does not allow scientists to tailor certain factors that would more faithfully represent a cell’s original environment. At Johns Hopkins University, Gregg Semenza’s lab discovered the first of many transcription factors that are induced by hypoxia, and followed up on that
work to find that oxygen levels are critical to the regulation of many genes from embryonic development through to processes performed by every adult cell, including cancer cells (Iyer). Since that initial discovery, many scientists have studied these hypoxia-inducible transcription factors, leading to a much richer understanding of how oxygen levels influence cell behavior.

Scientists at Xcell Biosciences have taken a new approach to cell culturing based on work in the cell biology field demonstrating the importance of specific hypoxia and pressure levels to cell microenvironments. By combining adjustable levels of oxygen and atmospheric pressure, they have developed a system that more accurately reflects a cell’s original home. Cancer cells, for example, respond well to 1% oxygen and about 2 pounds per square inch; skin cells, meanwhile, need 20% oxygen and just 1 PSI for ideal maintenance and fitness. Extensive studies have shown that even a small change in pressure or in oxygen level can be enough to stress a cell, altering its gene expression in a matter of hours.

Xcell Biosciences’ instrument, the Avatar System, allows scientists to customize oxygen, pressure, temperature, and CO₂ levels to recreate a cell’s native conditions. The system has been proven in multiple studies to maintain these custom conditions for prolonged periods. Cells grown in the Avatar System demonstrate biological traits that more accurately match those seen in vivo. The instrument is much smaller than traditional incubators, allowing it to fit under a sterile laminar flow hood. It also uses far less gas and provides a more streamlined workflow than is typical in cell culturing, allowing scientists to test cell growth under many different conditions to see which deliver the best results.

Results: Patient Tumor Cells Successfully Maintained in Culture

Several evaluations of the Avatar bioreactor demonstrate that it outperforms traditional incubators at keeping cells in their native habitat. Studies of gene expression in tumors indicate that cancer cells stored in standard cell culture do not produce the same expression pattern as the original tumor; in the Avatar system, cells produce expression profiles that match their in vivo source.

In one study, scientists isolated circulating tumor cells (CTCs) from prostate cancer patients and cultured them for a week with the Avatar platform (Xcell poster, ASCO 2016). Among other findings, the team noted elevated levels of CXCR-4 mediated signaling, a consistent theme among metastatic cancers but one that has not previously been detected in cancer cells maintained in a traditional incubator. The study also found that colonies grown from cells taken from the same patient produced different gene
expression patterns, indicating that the cells are not losing their tissue-specific identity during incubation.

Another demonstration used prostate cancer cell lines and peripheral blood mononuclear cells, stored under a range of hypoxia and pressure levels to investigate the resulting differences in gene and protein expression. Researchers found that under non-native conditions, the cells express different immunotherapeutic targets and activate pathways not typically found in vivo (Xcell poster, AACR 2015).

Applications for Primary Human Cells

An innovative bioreactor that allows users to customize pressure, oxygen, and other conditions to replicate native microenvironments is broadly useful for biological studies. For any kind of cell, more faithfully representing native biology will help scientists to generate and analyze more accurate genomic, molecular, and cellular data. An approach to culturing human cells that mirrors their in vivo persona offers a more useful alternative to animal models for goals such as personalized medicine, immunotherapy, therapeutic screening and testing, biomarker discovery, and more.

Conclusion

Traditional cell culture does not accurately represent the native microenvironment of cells, which has led to decades of ex vivo analysis of cells that do not behave as they would in nature. An innovative new way to culture cells factors in settings for pressure and hypoxia, more accurately mimicking the original cellular environment. Analyses show that cells stored under these conditions more closely mirror in vivo biology, potentially revolutionizing scientists’ ability to make meaningful discoveries from cultured cells.
References


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