transfection

A Tunable Microenvironment Improves Transfection Efficiency

One of the most promising and innovative areas of biomedical research involves isolating cells from a patient and then modifying and reintroducing them to that patient as a means of treating disease or regenerating damaged tissues. A key limiting factor in altering cells, however, is the inefficiency of introducing the desired material into cells through a process known as transfection. Coaxing cells to take up and express new genetic material — such as a DNA plasmid or other vector — to reprogram somatic cells for the creation of iPS cells or T cells for cancer therapy has high therapeutic potential but poor efficiency, usually with only a fraction of cells responding to the protocol.

The phenomenon of why cells seem so resistant to transfection is not fully understood. Factors contributing to successful transfection include the cell's natural ability to absorb genetic material, how much material is available to be taken up, how well cells can express the vector, and the viability of cells after transfection. What scientists know for certain is that transfection efficiency must be improved in order to achieve reliable, reproducible success in stem cell engineering, immunotherapy, and other cell-based therapies. With the growing potential for pairing transfection with gene editing tools such as CRISPR/Cas9 for generating genetically modified cells of interest, there is increasing demand to solve the transfection problem once and for all.

In a new study, scientists compared the results of transfection in two different cell culturing systems to determine whether environment plays a role in efficient uptake of genetic material. They directly compared a traditional incubator and a next-generation incubator designed to more accurately mimic the native microenvironment of any type of cell. This alternative technology, called the XPRESSR™ system and produced by Xcell Biosciences, uniquely incorporates custom settings for oxygen and atmospheric pressure in addition to the conditions included in standard incubators. For this study, scientists tested healthy and cancerous primary cells, which are notoriously difficult to transfect.

Study Results

While the problem at the root of efficient transfection may not be fully understood, scientists hypothesized that cells cultured in conditions more closely representing their native physiological environment might demonstrate better post-transfection viability and uptake of genetic material, resulting in higher plasmid expression. This project aimed to test that theory using the XPRESSR system, which in previous studies has been shown to outperform

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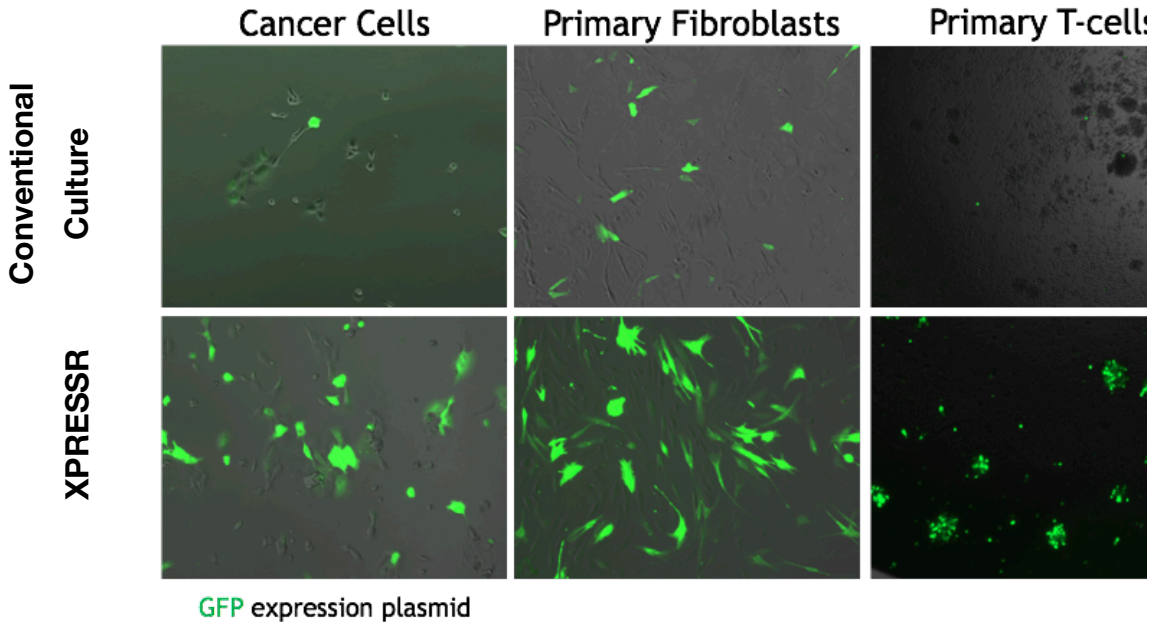
traditional incubators in maintaining cells at optimal fitness for long periods of time.

For this study, scientists used both electroporation and lipofectamine-based transfection with a plasmid containing green fluorescent protein (GFP) and a positive control for the CRISPR/Cas9 system. After transfection, cells were cultured either in a standard incubator or in the XPRESSR system tuned to physiologically relevant levels of oxygen and atmospheric pressure. Scientists also compared conventional serum-containing media to a human recombinant-based transfection media developed and optimized for primary cell culture by Xcell Biosciences.

Results were measured with imaging, scoring cells based on how many had incorporated the GFP construct and their relative brightness, as well as with qPCR to evaluate gene expression. Remarkably, all cell lines and primary cells showed higher efficiency in the XPRESSR system, with transfection efficiency improvements ranging from five-fold to 30-fold compared to their counterparts in the traditional incubator. The scientists also determined that it wasn't just the XPRESSR system that made the difference. Cells with the highest transfection efficiency were those cultured on the serum-free media in the XPRESSR; it appears that the combination of media and environmental conditions had a highly synergistic effect in producing the desired result.



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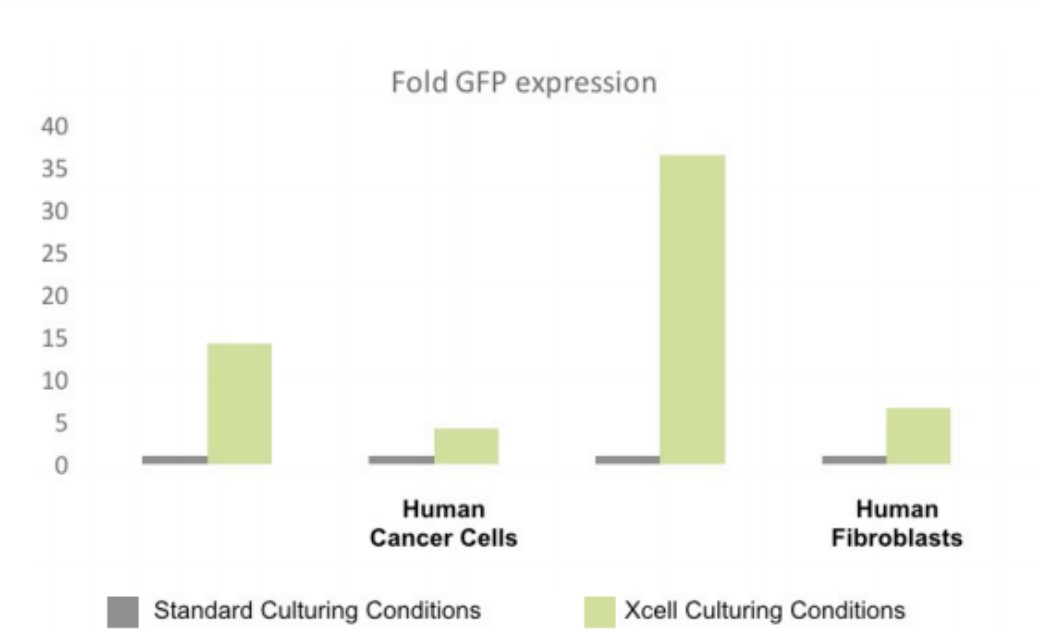

About XPRESSR

The XPRESSR system was developed in response to mounting evidence that traditional cell culturing methods do not maintain cells at optimal fitness levels, leading to discrepancies between biology measured in vivo and ex vivo. There is a growing body of research highlighting the importance of hypoxia and pressure to the cellular microenvironment. Scientists have shown that any modification in oxygen or hydrostatic pressure levels can be enough to change the gene and protein expression in cells.

By incorporating adjustable settings for oxygen and pressure levels in addition to the usual temperature and carbon dioxide levels, the XPRESSR system allows researchers to recreate the native microenvironment of their samples and to generate more biologically relevant results. Numerous studies of morphology, gene expression, proteomic profiles, and other factors have shown that cells stored in the XPRESSR system more faithfully reflect in vivo biology than cells stored in traditional incubators.

For cell transfection applications, the XPRESSR system allows users to culture cells across a range of physiologically relevant conditions to evaluate effects on function, phenotype, genotype, and more. With further studies using this system, it may be possible to determine the ideal conditions under which each cell type takes up and expresses new genetic material.

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Looking Ahead

This study demonstrates the utility of the XPRESSR system for optimizing transfection efficiency and indicates opportunity for other applications within cellular engineering and regenerative medicine. While these results by no means suggest a solution to the transfection challenge, they are a promising indication that it is possible to enhance and control transfection efficiency. It appears that microenvironment and media are two important factors for boosting this efficiency, but there will no doubt be many others to test in future research.

Transient transfection is a staple of cellular research. Although the study described here was geared toward understanding transfection as it relates to cell-based treatments, the same premise holds and could have dramatic implications for basic research in any number of applications. In the clinical realm, improving the efficiency of transfection could significantly boost success rates for patients treated with immunotherapy, stem cell engineering, and many other cell-based therapeutics.

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