Optimal Conditions for Embryonic and Neural Stem Cell Culture

Application Note

Growing stem cells in culture is a delicate balancing act between providing an optimal environment for maintaining pluripotency and guiding development down specific pathways when desirable.

In recent years, there have been significant advances in cell culture techniques for a variety of stem cells, including the development of sophisticated culture media, and novel transfection reagents for the genetic modification of stem cells.

In this article, we focus on several exciting new developments in stem cell culture techniques.

Feeder-free embryonic stem cell culture

Human pluripotent stem cells (hPSCs), which include embryonic stem (ES) cells and induced pluripotent stem cells (iPSCs), are remarkably difficult to maintain in an undifferentiated state in culture. hPSCs are unique in their ability to replicate and maintain an undifferentiated, pluripotent state indefinitely, so long as their environment provides the right mix of growth factors, cytokines and extracellular matrix proteins.

The first successful human ES cell culture was reported in 1998, when Thomson et al. reported the establishment of ES cell lines derived from human blastocysts (Thomson et al, 1998). In this initial paper, and for many years to follow, human ES cells were grown on a layer of mouse fibroblasts, known as the feeder layer of cells. Feeder cells were essential for maintaining ES cells in an undifferentiated state, as they provided an extracellular matrix for physical contact, as well as secreting necessary growth factors and cytokines that ES cells relied on for in vitro survival (Villa-Diaz *et al*., 2013). ES cell culture also traditionally used serum as a media supplement.

Although this approach was successful for establishing the first ES cell lines, the presence of non-human culture ingredients increases the risk of introducing adventitious agents into the cultures. As hPSC-derived therapeutics move from bench to clinic, it is increasingly important to move towards animal-free, or at least xeno-free, culture conditions to increase the safety of cell-based therapeutics.

The solution to this problem has been the introduction of feeder-free cell culture media that provides a defined, xeno-free growth environment for the feeder-free maintenance of pluripotent stem cells. Feeder-free media, such as MTI-GlobalStem's PluriQ™ G9, are utilized in conjunction with cell culture plates coated with an extracellular matrix, such as vitronectin. PluriQ™ G9, when used in combination with specific, high-quality growth factors, has been qualified for reprogramming somatic cells into pluripotent cells *in* vitro, and for expanding and maintaining pluripotent human ES and reprogrammed iPS cells (Figure 1).

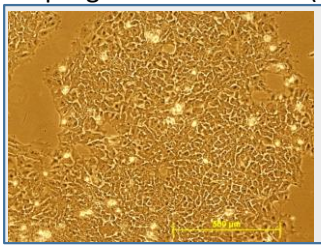

Figure 1. PluriQ™ G9 feeder-free cell culture medium supports maintenance in culture of human pluripotent stem cells. Human iPSCs shown here were passaged onto vitronectin in PluriQ™ G9 medium and expanded for 4 days. Multiple passages produced similar results (data not shown).

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Long-Term Culture of Neural Stem Cells

Stem cells that are committed to a specific developmental lineage create their own cell culture challenges. For example, neural stem cell cultures require one set of culture conditions to maintain long-term growth without losing multipotency over multiple passages, while committed progenitor cells require a different set of culture conditions to differentiate into specific neural cells. Not surprisingly, the quality and components of cell culture media are important factors in determining cell health and fate.

When culturing neural stem cells, it is important to not only maintain an undifferentiated state for the desired amount of time, but control, as much as possible, the differentiation pathway. For example, MTI-GlobalStem's **NeuralX™ Neural Stem Cell (NSC) Medium** is a basal medium formulated for maximum growth and survival of proliferative neural cells such as human iPS-derived neural stem cells or rat primary oligodendrocyte precursors. It is intended for use with **GS22™ Neural Supplement**. When combined to form a complete medium, NeuralX™ and GS22™ demonstrate superior performance in supporting the growth and survival of proliferating neural stem and progenitor cells in culture (Figures 2 and 3).

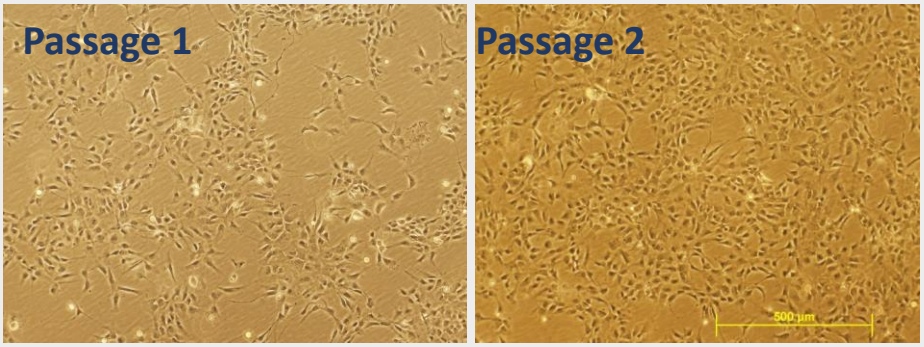

Figure 2. NeuralX™ Neural Stem Cell Medium and GS22™ Supplement support healthy growth of proliferating human iPSC-derived neural stem cells in culture over multiple passages. HIP™ Human iPSC-derived pluripotent neural stem cells (BC1 line;) were maintained in NeuralX™ Neural Stem Cell Medium and GS22™ Supplement for 4 days before passaging and continuing culture for another 4 days.

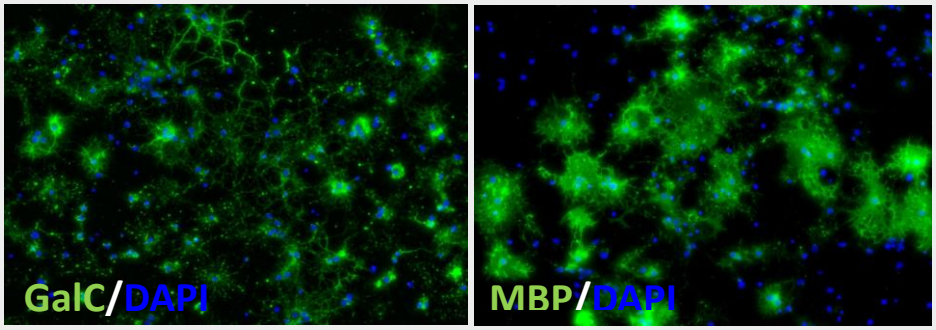

Figure 3. Rat primary oligodendrocyte precursors (GRPs; GSC-9430) differentiate into healthy, mature oligodendrocytes after initial growth in NeuralX™ Neural Stem Cell Medium and GS22™ Supplement. After growing rat GRPs in in NeuralX™ medium with GS22™ supplement for 3 passages, cells were switched to NeuralQ™ Basal Medium supplemented with GS21™ Supplement for 2 passages. Antibody labeling revealed a significant number of mature, differentiated oligodendrocytes, as indicated by labeling with GalC (green, left panel) and MBP (green, right panel). Total cells are shown using DAPI (blue, both panels).

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Genetic Modification of ES and Neural Stem Cells

Modifying gene expression is more difficult in some cell types than others. While it is well established that non-proliferating cells, such as post-mitotic neurons, are difficult to transfect, some stem cells have proven to be relatively resistant to genetic modification. Embryonic stem cells are relatively easy to transfect; however, more committed adult stem cells are more resistant. Transfection frequently alters cell viability and growth, even of proliferating stem cells.

One approach towards improving transfection efficiency is to use reagents specifically designed for stem cells. MTI-GlobalStem's **DNA-In™ Stem Transfection Reagent** offers a simple, robust and reproducible method to deliver DNA into human ES and induced pluripotent stem cells (iPSCs). Formulated and optimized specifically for embryonic and adult stem cells, DNA-In™ Stem is a new-generation transfection reagent that enables high efficiency transfection while maintaining maximum cell viability and cell growth. Recent data demonstrates that DNA-In™ Stem Transfection Reagent achieves maximum transfection efficiency and cell viability across multiple types of stem cells, requiring only low amounts of DNA for maximum expression and low toxicity (Figure 4).

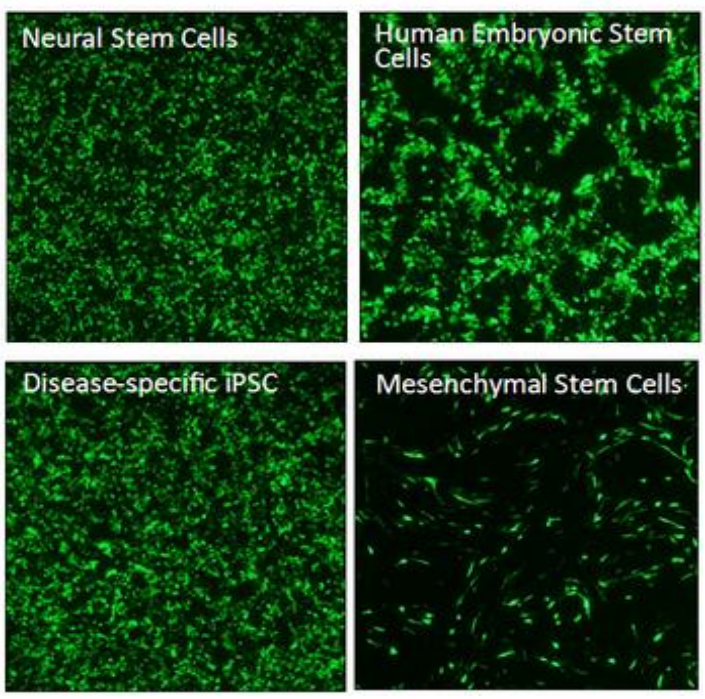

Figure 4. Cells transfected with DNA-In™ Stem Transfection Reagent. Cells were plated in 24-well plates to give 60-70% confluency on the day of transfection. Cells were transfected with various amounts of a DNA plasmid, containing eGFP driven by the EF1-alpha promoter using 1µl DNA-In™ Stem. Cells were observed 24-hours and 48-hours (not shown) post-transfection. Representative images of normal human iPSC-derived neural stem cells (NSC), Human embryonic stem cells, adipose-derived mesenchymal stem cells (MSC) and Huntington's Disease (HD)-specific induced pluripotent stem cells (iPSCs) show consistently high efficiency with low toxicity when using DNA-In™ Stem transfection reagent.

A second approach to increasing the efficiency of modifying protein levels in a cell is the introduction of mRNA directly into a cell. This approach has many advantages, including promoter-independent expression; faster protein expression following transfection; and no risk of insertional mutagenesis. **mRNA-In™ Stem** is a new mRNA transfection reagent specifically designed and optimized for high efficiency mRNA delivery with exceptionally low cytotoxicity in stem cells and hard-to-transfect primary cells. Unlike other reagents, mRNA-In™ Stem requires very low amounts of RNA to achieve maximum delivery while maintaining optimal cell viability (Figure 5).

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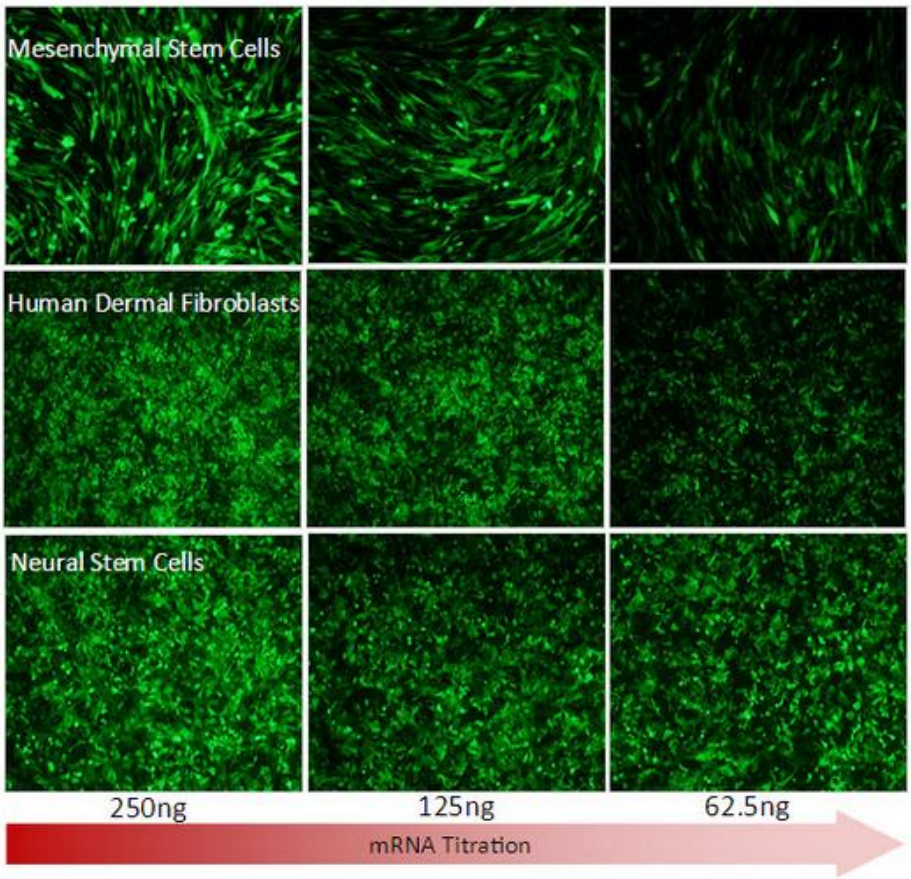

Figure 5. mRNA-In™ Stem achieves maximal transfection efficiency and expression with low amounts of mRNA. Human ES and iPSC-derived stem cells were plated in 24-well plates to give 60-80% confluency on the day of transfection. Low amounts (250ng to 62.5ng) of GFP-mRNA containing 5-methlycytosine and pseudouridine were complexed with 2-3µl mRNA-In™ in Opti-MEM®. Shown above are representative images of human adipose derived mesenchymal stem cells and normal human iPSC-derived neural stem cells (NSC) 24-hours post-transfection.

Summary

Recent advances in stem cell culture and transfection have opened up new approaches to studying stem cell biology. The ability to grow stem cells in xeno-free, feeder-free culture conditions enables the movement from bench to clinic, while advances in modifying gene expression allow the manipulation of stem cells for a variety of applications.

References

Thomson, J.A., Itskovitz-Eldor, J., Shapiro, S.S., Waknitz, M.A., Swiergiel, J. J., Marshall, V. S. and Jones, J. M. (1998). Embryonic Stem Cell Lines Derived from Human Blastocysts. *Science, 282*(5391), 1145-1147. DOI:10.1126/science.282.5391.1145

Villa-Diaz, L. G., Ross, A. M., Lahann, J., & Krebsbach, P. H. (2013). The evolution of human pluripotent stem cell culture: from feeder cells to synthetic coatings. *Stem Cells (Dayton, Ohio)*, *31*(1), 1–7. doi:10.1002/stem.1260

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