MTI-GlobalStem

Application Note

It is widely accepted that the reproducibility of data derived from cells in culture is heavily dependent on the quality of the culture medium, including any additives used to promote cellular viability and other key characteristics.

Lot-to-lot variability, contaminants in media and media additives, and even water quality of media can significantly change the culture qualities and experimental results.

Stem cells are particularly sensitive to media composition.

Even trace amounts of contaminants, such as endotoxin, can lead to altered biological responses in stem cells [1].

This paper examines key information regarding the impact of media additive quality on stem cell characteristics *in vitro*.

Impact of Media Additives on Stem Cell Behavior in Culture

The Need for Defined Cell Culture Media for Stem Cell Applications

Maintaining stem cells in culture requires a delicate balancing act between promoting cellular self-renewal and preventing or directing differentiation. Embryonic stem cells, induced pluripotent stem (iPS) cells and adult stem and progenitor cells all face a similar balancing act when cultured *in vitro*.

As our knowledge of stem cell biology has increased, the cell culture process itself has become more refined over time to exert greater control over the culture environment. Stem cells are highly sensitive to environmental cues, which dictate the timing and even the pathway of differentiation into mature cell types.

The diversity of stem cells is so great that there is no single cell culture medium that can support all types of stem cells, and many embryonic stem cells rely on a feeder cell system to provide a microenvironment that prevents stem cell maturation and differentiation[2]. However, as stem cells move from research to clinic, there is increasing need to develop more defined culture conditions, including animal-free, chemically-defined cell culture media supplemented with additives such as growth factors or cytokines.

When considering cell culture media in a regulated environment, it is necessary to examine a similar set of variables as biopharmaceutical manufacturers that utilize cell-based protein production systems. Cell culture medium and additives must have minimal lot-to-lot variability; be animal-free; and produce no undesirable side effects as a result of the quality of the culture ingredients themselves. The quality of cell culture ingredients is heavily dependent on the manufacturing methods of the ingredients, and customers rely on manufacturers to consistently produce and test the safety and reliability of these components.

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Comparing Additives Across Manufacturers

Companies manufacture cell culture media ingredients in different ways. For recombinant proteins, such as cytokines and growth factors, the acceptable lot release criteria vary significantly across manufacturers. For example, the release criteria for mouse leukemia inhibitory factor (mLIF) typically includes testing for bioactivity and sterility. The standard bioactivity assay for mLIF is the murine M1 myeloid leukemic assay[3], which tests the ability to induce differentiation of murine M1 myeloid leukemia cells (Figure 1). However, a more relevant bioassay for the purposes of stem cell research is the ability of mLIF to inhibit differentiation of mouse ES cells in culture. As shown in Table 1, some manufacturers rely solely on the murine M1 myeloid leukemic assay for mLIF, rather than taking the extra step of verifying activity in stem cells.

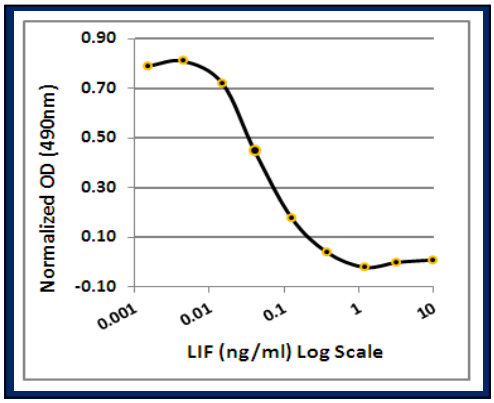

Figure 1. Proliferation bioactivity assay measuring ability of MTI-GlobalStem mLIF to induce differentiation of mouse M1 myeloid leukemia cells.

Table 1. Comparison of lot release criteria for mouse LIF (mLIF) from three leading manufacturers.

Manufacturer	Bioactivity - Murine M1 Myeloid Leukemia Cells	Bioactivity - ES Cell	Endotoxin Level	Tested for *Mycoplasma*	Contains carrier protein (BSA)
MTI-GlobalStem	Yes	Yes	<0.005 ng/µg	Yes	No
Company A	Yes	Yes	<0.01 ng/µg	No	Yes
Company B	Yes	No	<0.01 ng/µg	No	No

Another area of concern for media additives is sterility. Manufacturers routinely test for endotoxins, a type of lipopolysaccharide produced and released from bacteria such as *E. coli*, the most common bacteria used commercially for the production of recombinant proteins. As discussed below, endotoxin contamination poses a serious risk to cell culture, particularly stem cells. The currently accepted limit for endotoxin is 1 EU/µg, which has become the standard most manufacturers use as a manufacturing lot release criterion. However, as discussed below, stem cells are highly sensitive to even this low amount of endotoxin, making a lower testing limit more reasonable for this type of cell culture (Table 1). Independent testing by Charles River Laboratories of the endotoxin levels of growth factors in competitive growth factor products confirmed that other commercial growth factors typically contain up to 1 EU/µg

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endotoxin, while mLIF from MTI-GlobalStem is consistently at or below 0.005 EU/µg (data not shown).

Another contamination risk comes from *Mycoplasma*, an insidious cell culture contaminant that negatively impacts cell culture results and is very difficult to remove once contamination has taken hold. *Mycoplasma* typically is introduced to cell culture by contaminated cells, contaminated cell culture ingredients, or infected laboratory personnel. Cell culture ingredients are more likely to be contaminated if they contain an animal-derived ingredient, such as serum, bovine serum albumin (BSA) or trypsin. Although *Mycoplasma* testing is relatively easy, few manufacturers take the additional step of testing for *Mycoplasma* in their mLIF as a safety precaution for customers (Table 1).

Case Study: Effects of Endotoxin on Stem Cell Culture

Endotoxins are typically found on the outer cell wall of gram-negative bacteria, such as *E. coli*. When Gram-negative bacteria are used to produce recombinant proteins, endotoxins can sometimes carry over in to the final protein product. Endotoxins can also be introduced into cell culture media through the use of animal-derived products, such as serum and BSA. Endotoxins are known to have negative effects on a variety of cell culture parameters (Table 2).

The commonly accepted "safe" level limit of endotoxin in cell culture media is 0.1 ng/µg. As a result, most manufacturers use this as the cutoff for lot acceptance. However, there is increasing evidence that much lower levels of endotoxin significantly impact stem cell cultures.

Table 2. Published effects of endotoxin on cell cultures.

Enodtoxin Level	Observed Effect	Reference
0.01 pg/ml	Altered physical behavior of endothelial cells	Unger *et al*., 2014
2 pg/ml	Direct effect on myeloid progenitor cells; alters hematopoeitic cells in culture	Rinehart *et al*., 1997
5 pg/ml	Inhibition of directed differentiation of hESC to mesoderm	Sivasubramaniyan *et al*., 2008
100 ng/ml	Overwhelming evidence that cells are clearly affected by endotoxin concentration above 100 ng/ml	Gorbet *et al.,* 2005

In a study on embryoid bodies (EBs), endotoxin exposure as low as 0.005 ng/ml significantly inhibited stem cell differentiation into mesoderm[1]. Even lower concentrations (0.002 ng/ml) alter cytokine production of myeloid progenitor cells (MPCs), thereby altering the proliferative capacity of hematopoietic stem cells in culture[4]. This concentration correlates to adding a single growth factor containing 0.1 ng/µg (1 EU/µg) bacterial endotoxin to stem cell culture, assuming 20 ng/ml concentration of growth factor is utilized.

In another study, endotoxin levels as low as 0.001 ng/ml (equivalent to using a typical mLIF concentration of 10 ng/ml in culture) induced NANOG expression in mouse ES cells[5] (Figure 2).

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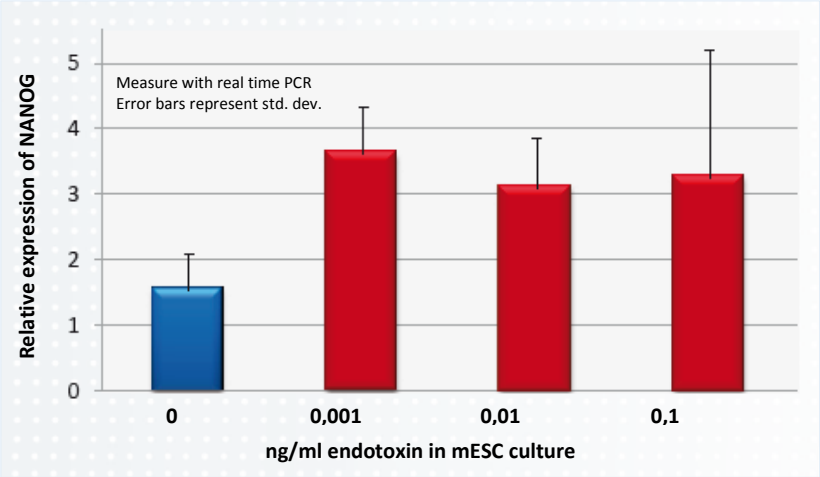
According to these results, endotoxin levels in commercial growth factors, currently regarded as acceptable, may significantly affect stem cell status and compromise stem cell maintenance and differentiation.

Figure 2. NANOG expression is significantly affected by currently accepted endotoxin levels in mESC cultures.



Source: Orf Genetics

Reproducible Results Using Better Additives

While this paper focused on mLIF as the exemplar, the argument that stem cell culture requires cell culture media and additives that are low in endotoxin, free of *Mycoplasma*, and produce reliably reproducible results, extends to other ingredients as well. As stem cell biology moves from bench to clinic, the availability of well characterized, safe ingredients becomes even more critical to the development of successful cell-based therapeutics.

References

1. Sivisubramaniyan K, *et.al*. Regen Med (2008) 3(1), 23-31.
2. Van Der Sanden B, *et al*. J Cell Biochem (2010) 111(4):801-807.
3. Metcalf, D, *et al*, Leukemia (1988) 2:216-221.
4. Rinehart J and Keville L. Cytotechnology (1997) 42:153-159.
5. Nolte J. http://www.orfgenetics.com/media/PDF/University_of_Gottingen_flyer.pdf

Supplier	Products	Content	Catalog No.
MTI-GlobalStem	Leukemia Inhibitory Factor (mLIF), carrier-free	10, 50 or 100µg/vial	GSR-7001
MTI-GlobalStem	Fibroblast Growth Factor, Basic (FGF-2)	50µg/vial	GSR-2001

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