ENHANCED CELL GROWTH WITH PHYSIOLOGICALLY RELEVANT MEDIA SUPPLEMENTS

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Abstract

Recent advances in genetic engineering have resulted in exponential growth in cell therapy technologies. This has led to a need for corresponding advances in the cell culture media that is used to recover, sustain and expand these important cells. Cell therapy researchers and manufacturers alike, have spent valuable time and money to optimize media formulations to enhance cell proliferation while maintaining functional capabilities of stem cells and immune cells. Proliferation rates and cell robustness often suffer in serum free media (SFM) or chemically defined formulations due to the lack of physiologically relevant protein sources and concentrations. Physiologix™ XF Human Growth Factor Concentrate (hGFC) is a cell culture media supplement that can be used in place of serum supplements with traditional basal media such as RPMI 1640 or DMEM/F12. In this study, hGFC was compared to various serum free media. $CD4^+$ T cells, bone marrow derived mesenchymal stem cells (BM-MSC) and induced pluripotent stem cells (iPSC) were cultured in the presence of hGFC or SFM. All cell types had preferentially higher cell count when grown in the presence of hGFC than in their corresponding SFM. In addition to this, $CD4^+$ T cell function was examined by looking at cytokine profiles, activation markers CD25 and CD69, and exhaustion marker PD-1. Cells showed higher for activation in presence of hGFC. They also exhibited greater ability for exhaustion under constant stimulation, which is indicative of a more physiologically relevant response to constant stress. The implications of this data are particularly important for those in cell therapy manufacturing where cell proliferation rate and viability are critical.

Introduction

- Valuable resources are spent optimizing cell culture media formulations (e.g. serum free or chemically defined) that are often prohibitively expensive or yield suboptimal cell growth.
- Safer and more reproducible media formulations are needed.
- FBS is no longer a viable option for translational stem cell and T cell work. Human serum suffers from lack of consistency.
- Cell therapy manufacturers need reagents that allow for them to proliferate cells in a safe and effective manner.

Physiologix™ XF Serum Replacement

Physiologix™ Xeno Free Human Growth Factor Concentrate is a novel serum replacement that is meant to replace fetal bovine serum (FBS) and human serum (HS) in conventional media formulations. It is particularly successful as a media supplement when used for stem cells and T cells. Past work shows that the optimal concentration of Physiologix™ XF is 2%.

Sourced from transfusion grade donor material, Physiologix™ XF SR has been through the following screens:

General:
- Sterility, Endotoxin, Mycoplasma
- pH and total protein

Functional proficiency:
- Seven day growth assay on bone marrow derived mesenchymal stem cells

Adventitious Agents:
- Human Immunodeficiency Virus (HIV) 1 & 2
- Hepatitis B & C Viruses
- Human T-Lymphotropic Virus Types I and II
- Treponema pallidum (Syphilis)
- Trypanosoma cruzi (Chagas disease)
- West Nile Virus
- Zika Virus

Physiologix™ XF is processed under cGMP conditions.

Experimental Design

BM-MSC: Bone-marrow derived mesenchymal stem cells were purchased from ScienCell Research Laboratories. Cells were passaged in DMEM/F12 media supplemented with 2% Physiologix™ XF hGFC or off-the-shelf "serum free" media. Proliferation was assessed using a standard Cell Titer Glo assay.

iPSC: Induced pluripotent stem cells were purchased from Thermo Fisher Scientific and passaged in DMEM/F12 media supplemented with 2% Physiologix™ XF hGFC or off-the-shelf "serum free" media. Proliferation was assessed using a standard Cell TiterGlo assay.

$CD4^+$ T Cells: PBMC were isolated from whole blood using a standard phase extraction protocol. CD4+ cells were isolated from PBMCs using bead-based negative selection kit and magnetic separation. Cells were then activated with anti-hCD3/28/2 and cultured for seven days in RPMI 1640 with Glutamax and 2% Physiologix™ XF hGFC or "serum free" media. Cells were stained with Ghost Dye 780 for viability and labeled with CFSE to assess proliferation. Cells were analyzed by flow cytometry.

For cytokine profile assessment, $CD4^+$ T cells were isolated and cultured as described above. Supernatant was collected on days 2,4 and 7. Multiplex bead based flow cytometry was performed on the supernatant.

Results

CD4+ T Cells

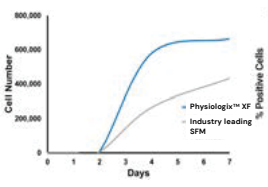

Figure 1. Representative data of $CD4^+$ T cells grown in SFM or RPMI 1640 supplemented with 2% Physiologix™. The cells grown with Physiologix™ showed a higher proliferation rate and cell number compared to the SFM control over the seven day time period.

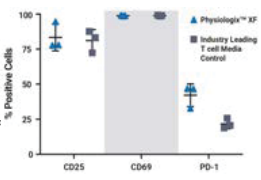

Figure 2. Surface marker expression after 7 days in off-the-shelf SFM or RPMI 1640 with 2%Physiologix™. Cells showed higher capability for activation (CD25 and CD69) in presence of Physiologix™ and exhibited greater exhaustion (PD-1) under constant stimulation, which can be more indicative of a more physiologically relevant response to constant stress.

Stem Cells

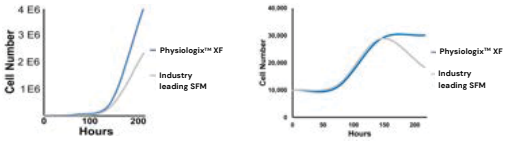

Figure 3. iPSC grown for 7 days in Physiologix™ showed higher cell count compared to the off-the-shelf SFM control.

Lot to Lot Consistency

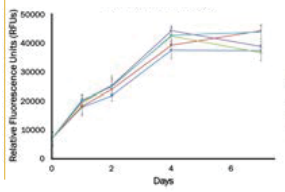

Figure 5. Standard DMEM/F12 was supple-mented with five different manufacturing lots and used to grow BM-MSCs for 7 days. Cells were assayed using a standard Cell TiterBlue Viability kit to yield a functional lot to lot comparison.

Cytokine	Control SFM	Physiologix™ XF hGFC	Trend Comments
IL - 2	++	++	Peaks at day 2
IL - 4	++	+++	Peaks at day 4
IL - 5	+	+++	Peaks at day 4
IL - 6	+++	+	Peaks at day 4
IL - 10	+	+	Peaks at day 2
IL - 13	++	+++	Peaks at day 4
INF_γ	d2:++/d4:++++	d2:++++	Peaks at day 2; Maxed out at day 2 for hGFC and at d4 SFM
TNF_α	+++	+++	Peaks at day 2

Table 1. $CD4^+$ cells were grown for 7 days under stimulation. Cytokine Profile from supernatant collected on days 2 and 4. Cells grown in the presence of Physiologix™ XF hGFC show more physio-logically relevant cytokine expression compared to cells grown in industry leading SFM.

Conclusions and Future Work

Conclusions

Media formulations using the consistent and more physiologically relevant Physiologix™ XF serum replacement outperform off-the-shelf SFM in the expansion and proliferation of iPSC, BM-MSC and $CD4^+$ T cells. This could be highly beneficial for those in cell therapy manufacturing where proliferation rate and consistency are critical.

Future Work

Cell number and growth rate are not the only characteristics of importance to those in cell therapy. Future work is aimed at further optimization of complete media formulations utilizing Physiologix™ XF SR. In addition, we are working with leading research centers to ensure that cells grown with Physiologix™ XF maintain phenotypic fidelity and functional capabilities (e.g. T cell durability and persistence).

Learn more!