

ENHANCED CELL GROWTH WITH PHYSIOLOGICALLY RELEVANT MEDIA SUPPLEMENTS

Lisa Karimi-Naser, Nucleus Biologics, LLC
 lnaser@nucleusbiologics.com
 Alyssa Master, PhD, Nucleus Biologics, LLC
 Stephen Orzell, Nucleus Biologics, LLC, USA

Key Words: T cells, stem cells, media supplement, cell therapy, gene editing

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. Serum free (SFM) and/or chemically defined formulations are thought to be superior due to the appearance that they are safer. However, proliferation rates and cell robustness often suffer due to the lack of physiologically relevant protein sources and concentrations. Therefore, there is a need for enhanced media supplements to help the cell therapy market thrive. 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 were isolated from the blood of

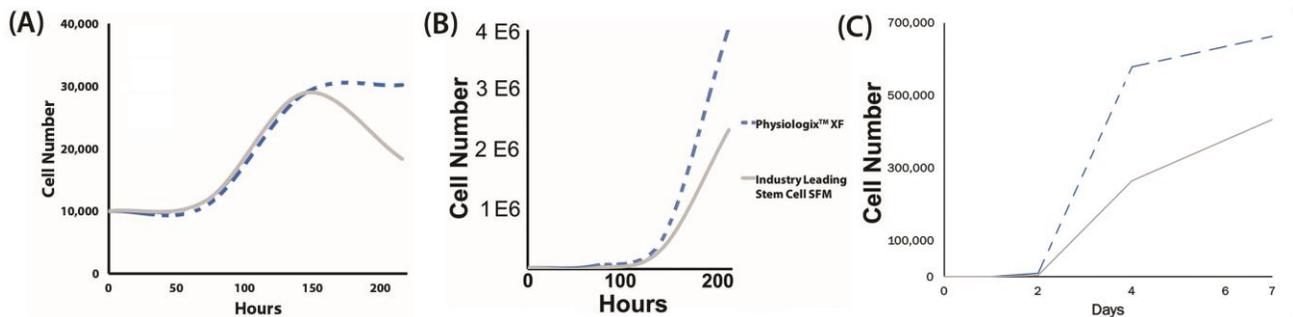


Figure 2. Proliferation of BM-MSK (A), iPSC (B) and CD4+ T cells (C) when grown for the indicated time in Physiologix supplemented media compared to industry leading serum free media.

three donors using standard protocol. Cells were labeled with CFSE and cultured over 7 days in RPMI 1640, Glutamax and 2% hGFC or SFM. Cells were collected on days 1,2,4 and 7, stained with Ghost Dye 780 for viability gating on flow cytometer and assayed using CFSE to determine proliferation. Bone marrow derived mesenchymal stem cells (BM-MSK) were purchased from ATCC. Culture conditions included DMEM/F12 supplemented with 2% hGFC or SFM. Cells were assayed using Cell Titer Blue. Induced pluripotent stem cells (iPSC) were purchased from Thermo Fisher Scientific. Culture conditions included Culture conditions included DMEM/F12 supplemented with 2% hGFC or SFM. Cells were assayed using Cell Titer Glo.

Figure 1 shows the difference in overall cell number after a week of culture in commercially available basal media supplemented with Physiologix™ XF hGFC. Figure 1A shows the BM-MSK grow in a similar fashion compared to commercially available SFM for the first few days after which the proliferation becomes markedly increased in the Physiologix™ condition. Similarly Figure 1B-C depicts a similar trend in iPSC and CD4+ T cells.

The implications of this data are particularly important

for those in cell therapy manufacturing where cell proliferation rate is critical. Cell growth is not the only characteristic of importance to those in cell therapy manufacturing. Future work is aimed at ensuring that cells grown in Physiologix™ XF media maintain phenotypic fidelity and functional capabilities.

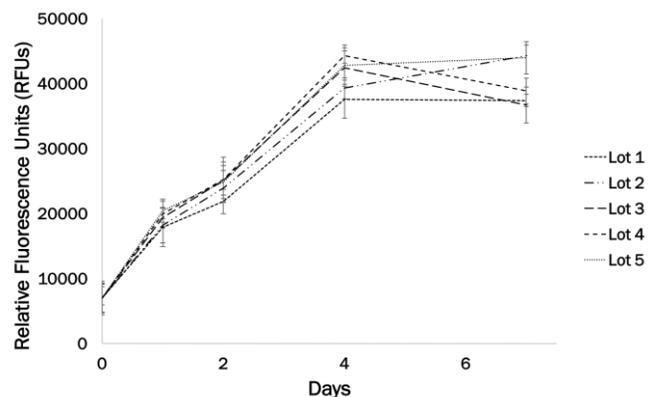


Figure 1. Proliferation of BM-MSK when grown in five different manufacturing lots of Physiologix™ XF hGFC supplemented media.