Benchtop to bioreactor: T-cell culture and expansion in chemically defined media

Anastassia A. Tselikova[1], Chandana Sharma[1], Robert Newman[1]
[1]Research & Development Department, FUJIFILM Irvine Scientific, Santa Ana, CA 92705

Introduction

Cell therapy is a blossoming field that focuses on the application of cells as therapeutic products; accordingly, the associated manufacturing process is cell culture. The difficulty of generating sufficient cell numbers with consistent clinical quality, a major challenge in the field, can be mitigated by addressing two factors: cell source and media composition. The removal of undefined media components is essential for a dependable, reproducible manufacturing process and safe translation for clinical use. Therefore, the development of cGMP-manufactured T-cell culture media that can be used reliably to culture, differentiate, and expand T-cells under serum-free conditions is essential. Following the FUJIFILM Irvine Scientific Rational Approach to media development, our extensive expertise has led to the creation of a chemically defined, animal-component free T-cell culture medium (CDM), with equal or better performance than other commercially available offerings while being cGMP grade.

Methods

Cell Source

PBMCs, positively- and negatively-selected CD3+ T-cells were provided by HemaCare. The cells were isolated from whole blood via leukapheresis using the Spectra Optia® Apheresis System, purified by density gradient centrifugation, and cryopreserved. The donors underwent an infectious disease screening panel and serological testing.

Cell Culture – Tissue Culture Plates and Flasks

Cells were seeded at 1x10^6 cells/mL in corresponding media + 200IU/mL IL-2 in 12-well plates. T-cells were activated either with 1µg/mL αCD3/αCD28 antibodies or a 1:1 ratio of activation beads to cells. Every 2-3 days, cells were counted and diluted with fresh, IL-2-containing media down to a concentration of 0.5x10^6 cells/mL. When necessary, cultures were transferred into larger vessel sizes (6-well plates, T-25 and T-75 flasks) to accommodate for increased volume and cell expansion.

Cell Culture – G-Rex® Vessels

In G-Rex 24-well plates, 1x10^6 cells were seeded in 7mL media + 200 IU/mL IL-2 and either 1µg/mL αCD3/ αCD28 antibodies or a 1:1 ratio of activation beads to cells. In 1L G-Rex bioreactor vessels, 33x10^6 cells were seeded in 1L media + 200 IU/mL and 1µg/mL αCD3/αCD28 antibodies. All G-Rex conditions were supplemented with 200IU/mL IL-2 every 2-3 days. Additionally, the 24-well plates had 75% of the media per well replaced with fresh complete media every 4-5 days.

Cell Analysis

Cell counts, viability, and average cell diameter were determined by Vi-CELL XR analysis. Flow data was gathered on the BD FACSVerse 6-color flow cytometer and analyzed with FACSuite software.

Results

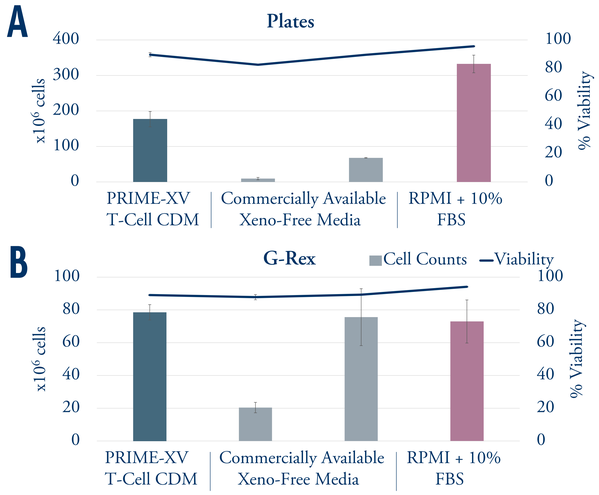

FIGURE 1. Total cell counts and viability of CD3+ T-cells activated using αCD3/αCD28 antibodies. Cells were activated using 1µg/mL each of αCD3 (Clone OKT3) and αCD28 (Clone CD28.2). Cells were analyzed on day 14 post-activation. (**A**) Cells cultured in CDM in 12-well tissue culture plates outperformed commercially-available XFM, but expanded less than RPMI + 10% FBS. (**B**) Cells cultured in CDM in 24-well G-Rex plates performed as well as or better than XFM and RPMI + 10% FBS.

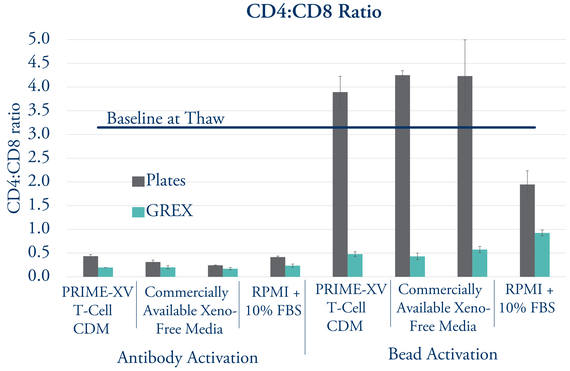

FIGURE 2. CD4:CD8 ratios of CD3+ cells at day 14 post-activation. CD8+ cell expansion was favored in all conditions except bead-activated plate culture conditions. Cells were activated with 1µg/mL αCD3 and αCD28 or a 1:1 ratio of activation beads, and cultured in 200 IU/mL IL-2.

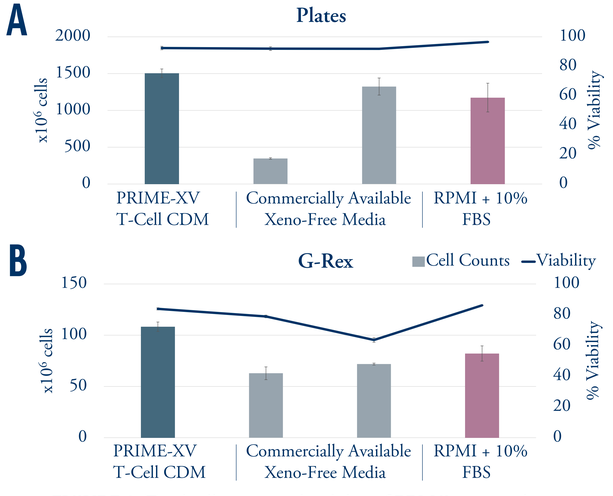

FIGURE 3. Total cell counts and viability of PBMCs activated using activation beads. CDM outperformed both XFM media and RPMI + 10% FBS. Cells were activated a 1:1 ratio of cells to activation beads. Cells were analyzed on day 14 post-activation. Cells were cultured in (**A**) 12-well tissue culture plates and (**B**) G-Rex plates as described in the "Methods" section of this poster.

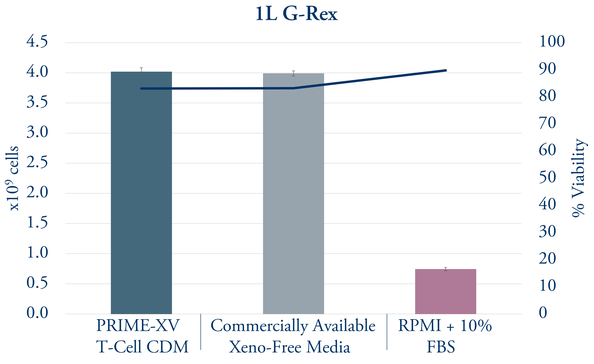

FIGURE 4. Total cell counts and viability of PBMCs in 1L G-Rex vessels. CDM and XFM equally outperformed RPMI + 10% FBS. Cells were activated with 1µg/mL αCD3 and αCD28 and cultured for 14 days in 200 IU/mL IL-2.

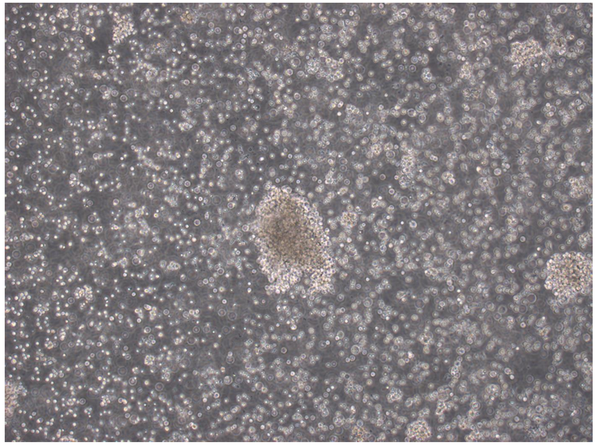

FIGURE 5. Representative image of expanded PBMCs at day 14 post-activation. Image taken at 100x magnification using an Olympus IX71 inverted phase contrast microscope.

Conclusion

PRIME-XV T-cell Chemically Defined Medium supports T-cell activation and expansion to the same degree or better than commercially available xeno-free media and, in most cases, better than RPMI + 10% FBS. Though magnitude of expansion and relative performance of benchmark media differed between cell donors, these results were representative of the trend seen across all runs.

Drawing from a vast registry of donors at HemaCare, we targeted peripheral blood mononuclear cells (PBMCs), positively selected CD3+ cells, and negatively isolated CD3+ cells, demonstrating the effectiveness of FUJIFILM Irvine Scientific's PRIME-XV T-cell CDM using multiple cell culture systems and activation methods. HemaCare's precisely isolated and purified cells cultured in PRIME-XV T-cell CDM maintained robust expansion, viability, and phenotype trends across donors and cell culture systems, from small-scale plates and flasks to scaled-up systems. Future projects will focus on expanding to additional bioreactor systems.

Acknowledgements

We would like to sincerely thank HemaCare for providing the cells and Dr. Dominic Clarke for his support.