Determining Optimal Seeding Density for Human T Cell Expansion in G-Rex

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Introduction

Efficiently expanding human T cells in a closed-system while maintaining a consistent cell phenotype is an active challenge for autologous and allogeneic T cell therapy manufacturers. Cell phenotype drift and variability in cell yield are common pain-points when transitioning from research protocols (i.e. flask or well plates) into larger scale, closed manufacturing systems (i.e. gas permeable bags, stirred tank bioreactors). This variability can be partially attributed to the introduction of motion and liquid media exchanges to accommodate cell expansion within larger volumes. G-Rex bioreactors have been shown (e.g. TIL, NK cells, T cells) to resolve these challenges by providing a static growth environment for cells, void of motion or media exchange, and promoting consistent cell expansion with reproducible cell phenotypes.

Optimizing cell expansion in G-Rex at small-scale is key to achieving efficient cell expansion. In this study we investigated the kinetics of human T cell expansion in small scale G-Rex6M bioreactors using ScaleReady reagents. Specifically, we determine the affect of seeding density on fold expansion and total cell yield. In addition, we look at the phenotypic composition of expanded T cells, including total CD4$^+$ and CD8$^+$ cells as well as memory CD4$^+$ T cells.

Methods

Human CD3$^+$ T cells from 3 donors seeded at two densities (0.5x10^6, 1.0x10^6) into G-Rex®6M Well Plates. For each donor, separate G-Rex6M Well Plates were seeded for undisturbed cell expansion until harvest and cell analysis at Day 0, 3, 6, 8, and 10. Triplicates for each donor were run at each collection timepoint. All cells were cultured using ExCellerate™ T Cell Expansion Media supplemented with GMP rhIL-7 and GMP rhIL-15. Cloudz™ T Cell Activation Kit was used for cell activation. Cloudz CD3/CD28 microspheres were added at Day 0 and removed at Day 2. After Cloudz removal, cell were returned to G-Rex 6M Well Plates for undisturbed rapid expansion using ExCellerate T Cell Expansion Media supplemented with IL-7 and IL-15. A detailed experimental procedure overview is depicted below.

Experimental Overview

Day 0 → **Day 2** → **Day 3, 6, 8, or 10**

Cell Activation
- Add 10mL ExCellerate T Cell Media to each well of a G-Rex6M
  - » GMP IL-7 (10 ng/mL)
  - » GMP IL-15 (5 ng/mL)
- Add CD4$^+$CD8$^+$ T-cells
- Add Cloudz

Rapid Expansion
- Harvest cells from G-Rex6M
- Dissolve Cloudz, Collect cells
- Add cells back to G-Rex6M
- Bring media to 100mL using 100 mL with ExCellerate T Cell Media plus IL-7 & IL-15

Harvest Cells

Optimal harvest day will vary pending type and culture conditions

Conditions

Condition	Cell Number	Cell Density	Cloudz Concentration	Checkpoint / Harvest Day	Glucose & Lactate Measurement
#1	5x10^6	0.5x10^6/cm^2	25 µL/1x10^6 cells	Day 3 Day 6 Day 8 Day 10	Day 6 Day 7 Day 8 Day 9 Day 10
#2	1x10^7	1x10^6/cm^2	25 µL/1x10^6 cells		

*Each condition was repeated with 3 donors

Raw Materials

Product Name	Concentration	Catalog Number	Supplier
G-Rex® 6M Well Plate	N/A	80660M	Wilson Wolf
GMP rhIL-7	10 ng/mL	207-GMP	Bio-Techne
GMP rhIL-15	5 ng/mL	247-GMP	Bio-Techne
Cloudz™ T Cell Activation Kit	25 µL/1x10^6 cells	CLD001	Bio-Techne
ExCellerate™ T Cell Media	100 mL/well	CCM030	Bio-Techne

Results

Similar Cell Expansion Kinetics and Cell Yields Across Seeding Densities

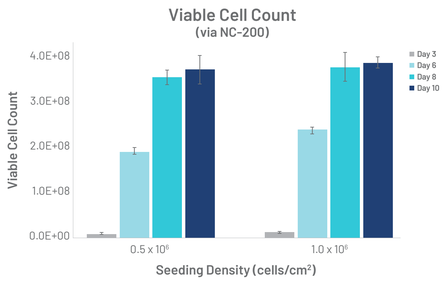

Figure 1: Low and High Seeding Density Resulted in Similar Final Cell Yield in G-Rex6M. Purified CD3$^+$ human T cells were seeded into G-Rex6M well plates at either 0.5x10^6 cells/cm^2 or 1.0x10^6 cells/cm^2 surface area. Viable cell counts were obtained at Day 3, 6, 8, and 10. Data show that by Day 8 in culture, lower seeding densities (0.5x10^6 cells/cm^2) achieve similar cell yields to wells seeded at a higher density (1.0x10^6 cells/cm^2).

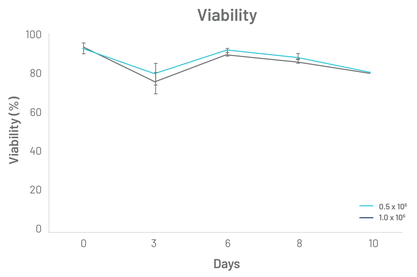

Figure 2: Robust Viability Throughout Culture Duration. Cell viability was determined at Day 0, 3, 6, 8, and 10 for cells seeded at 0.5x10^6 or 1.0x10^6/cells/cm^2. Under each condition, G-Rex6M cell viability of >80%. Cell viability trended downward at Day 10, corresponding to achieving maximal cell densities and plateauing levels of lactate in cell culture medium.

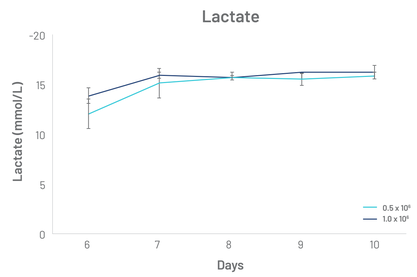

Figure 3: Lactate Measurements Predict Cell Expansion Kinetics. Lactate levels in cell culture media were measured on Day 6, 7, 8, 9, and 10. Plateuing levels of lactate are observed in G-Rex when cell reach maximum cell densities. We observed increasing lactate in cell culture media during rapid cell expansion (Day 7), as indicated in Figure 1. Lactate plateaus after Day 7 indicating slowing rates of expansion and maximal cell densities were achieved.

Higher Fold Expansion Using Lower Seeding Density

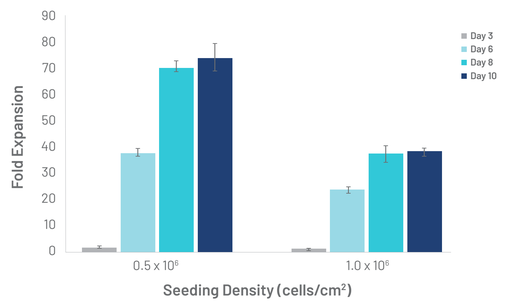

Figure 4: Lower Starting Cell Density Maximizes G-Rex Cell Expansion Capacity. Purified CD3$^+$ human T cells were seeded into G-Rex6M well plates at either 0.5x10^6 cells/cm^2 or 1.0x10^6 cells/cm^2 surface area. Fold expansion was calculated based on viable cell counts obtained at Day 3, 6, 8, and 10. Much higher fold expansion was observed for T cells seeded at a lower density (70-fold at Day 8) compared to the higher density (36-fold at Day 8). Taken together with cell yield data in Figure 1, these data show that maximum cell yield and expansion kinetics can be achieved using a lower starting cell density, therefore improving the efficiency of T cell expansion and minimizing donor cells needed for cell therapy expansion.

Results

High Fold Expansion of CD4$^+$ and CD8$^+$ Cells

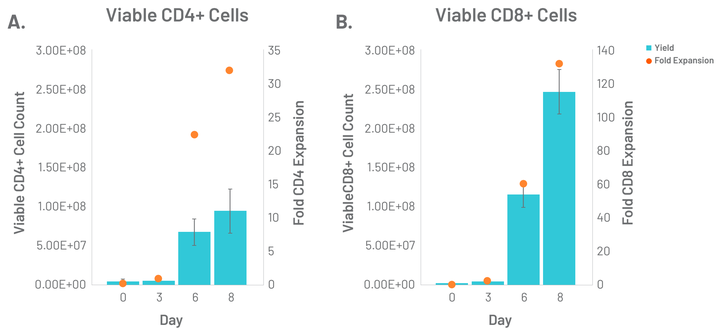

Figure 5: High Fold Expansion of CD4$^+$ and CD8$^+$ T Cells. G-Rex6M well plates seeded with 0.5x10^6 cells/cm^2 were evaluated for CD4$^+$ and CD8$^+$ T cell expansion at Day 0, 3, 6, and 8. **(A)** CD4$^+$ T cells showed robust fold expansion (33-fold; orange dots) with cell yields (blue bars) of 1x10^8 cells at Day 8. CD4$^+$ cells expanded rapidly between Day 3 and 6, with continued but slower expansion out to Day 8. **(B)** CD8$^+$ T cells showed robust fold expansion (118-fold, orange dots) with cell yields (blue bars) of 2.5x10^8 cells at Day 8. CD8$^+$ T cells expanded rapidly through Day 8 in culture.

Rapid Expansion of Memory CD4$^+$ T Cell Population

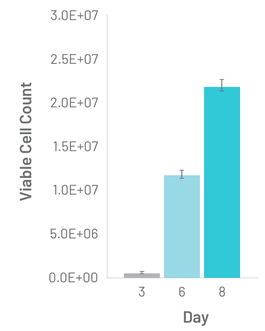

Figure 6: Rapid Expansion of Memory CD4 T Cells. G-Rex6M well plates seeded with 0.5x10^6 cells/cm^2 were evaluated for Memory CD4$^+$ T Cells at Day 3, 6, and 8. CD4 cells expression CD45RA$^+$/CCR7$^+$ or CD45RA$^-$/CCR7$^+$ were determined as CD4 memory T cells. CD4 memory T cells showed rapid expansion between Day 3 and 6, achieving 2x10^7 cells in a single well of a G-Rex6M well plate.

Conclusion

- Maximum T cell yield and expansion kinetics can be achieved using a lower starting cell density in the G-Rex. This improves the efficiency of T cell expansion by minimizing the number of donor cells needed to achieve maximum expansion within a G-Rex.
- G-Rex facilitates rapid expansion of T cells
- G-Rex T cell expansion is compatible with ScaleReady reagents:
  - » Cloudz T Cell Activation Kit
  - » GMP IL-7 & GMP IL-15
  - » ExCellerate T Cell Expansion Media
- Small-scale preliminary data suggests:
  - » Successful expansion of pre-selected CD4$^+$ and CD8$^+$ cells in a G-Rex6M Well Plate
  - » Seeding densities of 0.5x10^6 cells/cm^2 and 1x10^6 cells/cm^2 produced 3.7x10^8 and 3.8x10^8 amount of total viable cells, respectively, with > 85% viability.
  - » Desired yield, phenotype, and composition of final product may dictate harvest date.

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ScaleReady is a Joint Venture formed by Bio-Techne, Fresenius Kabi, and Wilson Wolf. Combining selected offerings from the three partners, the ScaleReady manufacturing platform combines tools and technologies for cell culture, cell activation and expansion, gene editing, and cell processing.

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