T cell expansion in PBS Mini vertical-wheel bioreactor using CellGenix® reagents

Introduction

Cell and gene therapies continue to be the fastest-growing area of therapeutics, with several new therapies available to patients and hundreds more in development. Sustaining such innovation requires biopharma companies to solve many operational challenges, particularly in manufacturing and scaling up therapies. Cell and gene therapies typically require a substantial amount of cells per dose, which means that the bioreactor-based expansion process of therapeutically active cells is key. The PBS bioreactor systems have been demonstrated to be suitable for the cultivation of mesenchymal stem cells and induced pluripotent stem cells. The vertical-wheel of the scalable bioreactor offers efficient fluid mixing while minimizing shear forces. To date, there is no protocol available for T cell expansion using PBS bioreactors. This study is a first of its kind and focused on the cultivation of T cells using CellGenix® GMP serum-free and xeno-free T cell medium (TCM). CellGenix® GMP TCM is a ready-to-use medium that needs no supplementation with glutamine or human serum.

The PBS Mini vertical-wheel bioreactor, together with CellGenix® GMP TCM supplemented with CellGenix® GMP IL-7 and CellGenix® GMP IL-15 enables T cell expansion to high cell densities and yields under gentle conditions. The final cell product contains no activation beads and the cells have an early-differentiated central memory phenotype. Our studies show that CellGenix® GMP products work well both in the G-Rex system as well as PBS bioreactors and T cell expansion and viability are comparable in both systems. The vertical wheel rotation did not affect the quality and functionality of CellGenix® GMP TCM, and GMP cytokines.

Methods

$CD3^+$ T cells of two human donors were purified using a negative selection kit, as purified T cells lead to very reproducible expansion results. CellGenix® GMP IL-7 (10 ng/ml), CellGenix® GMP IL-15 (10 ng/ml), Pen/Strep (1:100), and 0.1 % (v/v) Pluronic F68 were added to pre-warmed CellGenix® GMP TCM and 5 ml were filled into a G-Rex® 6 well plate. T cells were polyclonally activated using Dynabeads™ Human T-Expander CD3/CD28 (Gibco) in a 1:3 cell-bead ratio and seeded into the pre-filled G-Rex® at a cell density of 2.5×10^5 $CD3^+$ T cells/cm^2. Cells were cultured for 3 days and either all cells or the indicated number were transferred to a PBS Mini 0.1 MAG vessel containing 60 ml fresh medium. The rotation speed was set to 20 rpm unless otherwise specified. Cells were cultured up to day 10 with a 40 ml media addition on day 5. As a control condition, the T cells from pre-cultivation were transferred into a G-Rex® 6M plate and cultivated as described for the PBS Mini bioreactor. T cell yield, expansion, viability via 7AAD staining, and phenotype were determined by flow cytometry. The results of one representative donor shown in the following experiments were reproduced with a second donor and led to comparable results.

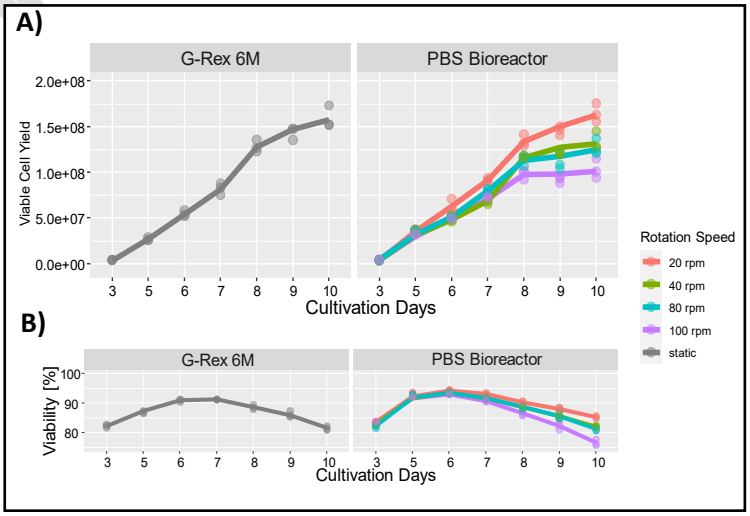

Figure 1: T cell yield and viability in PBS Mini bioreactor at 20 rpm condition are comparable to cultivation in G-Rex® 6M

CD3+ T cells purified from a human donor (n=1) were cultured as described with CellGenix® GMP TCM. Viable cell yield (A) and viability (B) were determined by flow cytometry in technical triplicates.

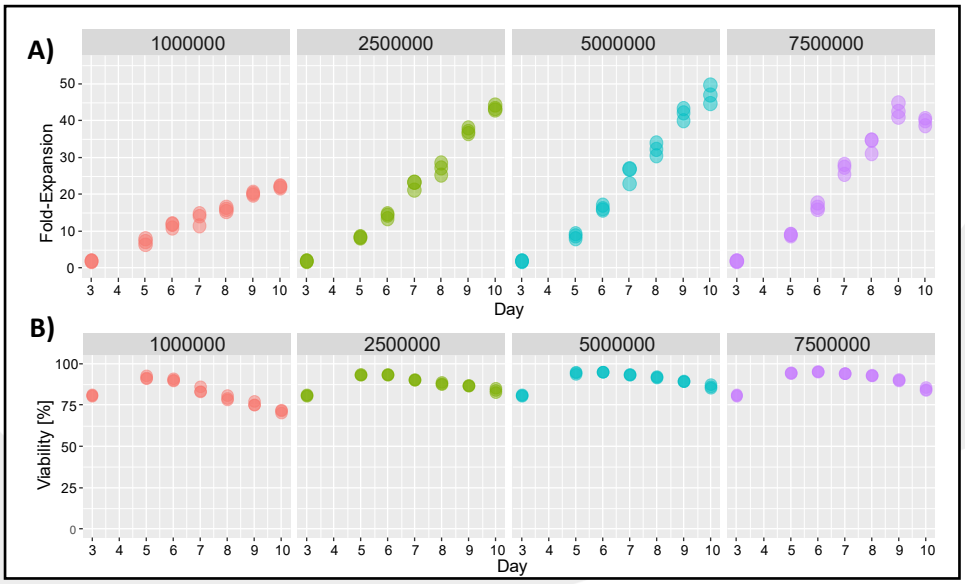

Figure 2: T cells expand to a comparable extent at various seeding densities
CD3+ T cells purified from a human donor (n=1) were cultured with CellGenix® GMP TCM as described. Different cell numbers from a cell pool were transferred into PBS 0.1 MAG vessels. Expansion of viable T cells (A) and viability (B), shown for each seeding density, were determined by flow cytometry in technical triplicates every day.

Results

PBS bioreactor requires very low rotation speeds for high T cell yields

Cultivating human cells in stirred bioreactor systems can lead to high shear stress levels for the cells. Thus, different rotation speeds in the PBS Mini bioreactor were compared to the static G-Rex® system. Cultivating the T cells at 20 rpm led to the highest mean T cell yield of $1.6x10^8$ cells in total at day 10, whereas increased rotation speeds lowered the mean T cell yield to $1.0x10^8$ cells at 100 rpm (Fig. 1). The mean T cell yield in the G-Rex® control was comparable to the 20 rpm condition with $1.5x10^8$ cells. T cell viability remained at 85% at high levels in the 20 rpm condition and was even higher as in the G-Rex, but decreased with increased rotation speed showing the negative effect of shear stress. Based on these results, the rotation speed was set to 20 rpm for the following experiments.

High T cell expansion rates at multiple seeding densities

High variability in the amount of initial starting material makes it difficult to obtain robust cell expansion in cell therapy development and manufacturing. To evaluate the optimal seeding range for fast and robust expansion in the PBS bioreactor, different cell amounts were transferred on day 3 into a PBS 0.1 MAG vessel. Starting with $2.5x10^6$ to $7.5x10^6$ T cells in 60 ml led to a comparable T cell expansion until day 10 between 47-fold and 55-fold with high viabilities of 85 %. With the highest seeding density of $7.5x10^6$ T cells, very high cell densities of $3x10^6$ T cells/ml were achieved. Seeding only $1x10^6$ T cells in 60 ml led to a 31-fold expansion with early decreasing viability to 70 % until day 10 (Fig. 2). This indicates that low seeding densities can be very critical for the survival of the cells whereas robust expansion can be achieved with more starting material. We recommend a protocol that starts with $2.5x10^6$ T cells in the G-Rex for activation and a transfer of $2.5x10^6$ to $7.5x10^6$ T cells into the PBS Mini Bioreactor.

High proportion of CD8+ T cells with a central memory phenotype

The phenotype of the final cell product is crucial for the clinical outcome. $CD8^+$ T cells are important for tumor killing and a memory phenotype is important for long-term persistence in the patient. The T cells cultivated at a rotation speed of 20 rpm had a high proportion of $CD8^+$ T cells. The majority of both, the $CD8^+$ and $CD4^+$ T cells, had a central memory phenotype with low expression of the exhaustion marker PD-1 (Fig. 3). This indicated that the phenotype of the final cell product was not altered by the bioreactor conditions.

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PBS Mini bioreactor removes magnetic activation beads automatically

The vertical-wheel impeller in the PBS 0.1 MAG vessel contains magnets that drive the rotation. For the activation of T cells, magnetic activation beads are widely used. These beads must be removed from the final cell product which requires an additional process step. The magnet in the vertical-wheel overcomes this problem by removing the beads very efficiently during the cultivation process (Fig. 4).

Another benefit of the bead removal is that the time of activation of the T cells can be controlled. This means that as soon as the T cells are transferred to the PBS bioreactor , the activation beads have less or no contact with the T cells.

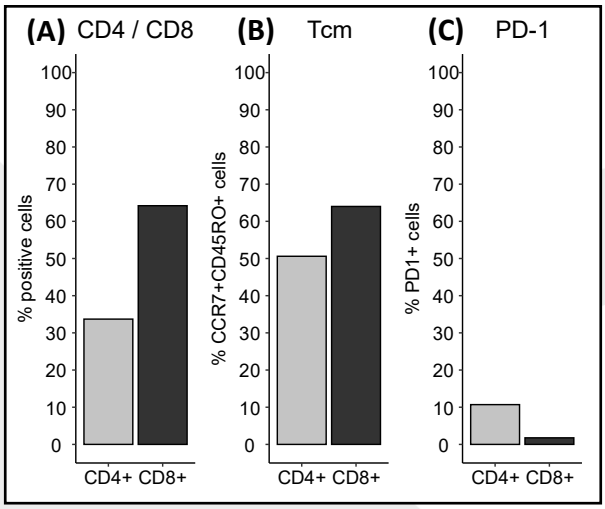

Figure 3: The CD4⁺ and CD8⁺ phenotype of the final cell product was not altered by the bioreactor conditions

The phenotype of the 5x10⁶ seeding condition (see Figure 2) was characterized by flow cytometry. **(A)** Percentage of CD4⁺ and CD8⁺ T cells in the total cell population which represent the two main T cell subtypes. **(B)** Central memory T cells (Tcm) were defined as CD45RO⁺CCR7⁺ T cells for CD4+ and CD8+ T cells. **(C)** PD-1 is a surface marker for T cell exhaustion and was analyzed for CD4+ and CD8+.

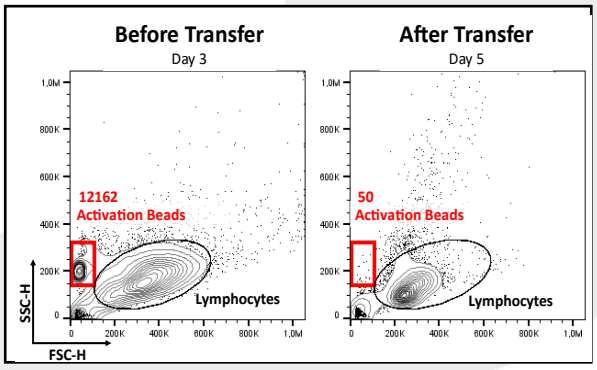

Figure 4: PBS Mini bioreactor removes magnetic activation beads

The number of magnetic activation beads was determined by flow cytometry before the cells were seeded in the PBS Mini bioreactor and after two days of cultivation.

Summary

Successful cell and gene therapy manufacturing depend on high-quality raw materials (ancillary materials according to the USP). Sartorius CellGenix offers high-quality GMP-grade raw materials that perform well in stationary conditions as well as in bioreactors. The PBS Mini bioreactor, together with CellGenix® GMP TCM supplemented with CellGenix® GMP IL-7 and CellGenix® GMP IL-15 is a suitable system for the cultivation of T cells. Low rotation speeds together with no need for an extra debeading step at the end of cultivation lead to high amounts of viable T cells with a favorable phenotype. We recommend a protocol that starts with 2.5x10⁶ T cells in the G-Rex for activation and a transfer of T cell population between 2.5x10⁶ to 7.5x10⁶ into one PBS Mini Bioreactor. The starting volume should be at least 60 ml to avoid foam formation. Feeding can be adjusted to the process but seems not to be critical for T cell expansion in the 100 ml culture. When scaling up in larger PBS bioreactors media addition or exchange should be considered. Our study highlighted that CellGenix GMP products are suitable for any cultivation conditions, static and rotatory, and ensure a high yield of fully viable and functional T cells.

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