T Cell Expansion in VueLife® "HP" Series Bags using Culture Clamps

VueLife® "HP" Series Bags are Saint-Gobain Life Sciences' "high-permeability" FEP bags for *in vitro* bioprocessing applications. Applications include the culture of highly concentrated immune cells, such as T or NK cells that require higher gas exchange rates for efficient culture and/or expansion *in vitro*.

The novel "HP" FEP laminate provides elevated oxygen and carbon dioxide permeability to the container, thereby introducing further enhanced gas exchange capability to the VueLife® product line. The fluid contact-layer remains the same material, as used in the VueLife® "C" Series range, harnessing the superior material properties of FEP. Each "HP" Series bag typically features a needleless injection site and "Y" connector with 7" PVC tubing leading to a female luer and a heat sealed sterile docking tube. The rounded corners provide strength, durability, and maximal product retrieval.

EXPANSION OF T CELLS IN VUELIFE® "HP" SERIES BAGS

The goal of this study was to develop a protocol to expand human primary T cells in 200-HP bags. We also compared T cell expansion in VueLife® 197-C bags to 200-HP bags to investigate the impact of increased gas permeability on cell yield. T cells were phenotyped before and after culture to characterize the impact of culture in VueLife® bags on surface marker expression. Finally, culture media parameters like pCO_2, pO_2, pH, glucose and lactate were measured to better understand the cells' metabolic activity and changes in the culture microenvironment in the different culture containers and conditions.

MATERIALS AND METHODS

VueLife® Series Bags and culture clamps

This study was conducted using Saint-Gobain Life Sciences' VueLife® "HP" Series (200-HP) and "C" Series (197-C) Bags. These bag models were selected to ensure comparable bag dimensions and aspect ratios for fair comparison of cell performance in both systems. Prototype culture clamps were used to compartmentalize the bag to facilitate the use of flexible fill volumes for optimal cell expansion, as illustrated in Figure 1.

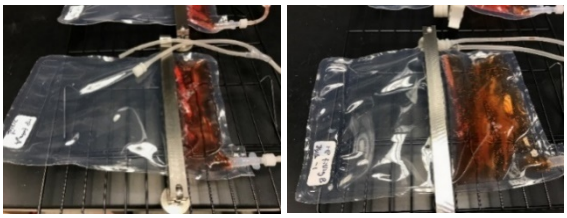

Figure 1. Compartmentalization of VueLife® bags using prototype culture clamps.

T cell isolation from Leukopak™

$CD4^+/CD8^+$ cells were isolated from fresh Leukopak™ apharesis products (Miltenyi Biotec) from healthy donors using Miltenyi Biotec's StraightFrom® CD4/CD8 MicroBead Kits and the MultiMACS™ Cell24 Separator Plus instrument.

T cell activation and expansion

Isolated $CD4^+/CD8^+$ T cells were resuspended in OpTmizer® media supplemented with L-Glutamine (200µM/mM, Gibco) and IL-2 (200 IU/mL, Miltenyi Biotec), and activated using Gibco™

CTS™ Dynabeads™ CD3/CD28 at a bead-to-cell ratio of 1:1. T cells were expanded for a culture period of 10-12 days and then harvested for characterization by flow cytometry.

Characterization of T cells

Beads were removed from cell samples prior to analysis using the DynaMag®. Cell counts and viability were determined using the Vi-CELL™ Cell Viability Analyzer (Beckman Coulter Inc.). This system utilizes the Trypan Blue dye exclusion method for quantification of viable cells in the measured cell population and provides results for mean cell size. The surface marker profile of generated T cell subsets was assessed via a BD LSR II flow cytometer (Becton Dickinson and Company) at the Flow Cytometry Core Facility, University of Massachusetts Medical School in Worcester, MA. The acquired data were analyzed using FlowJo v10 (FlowJo, LLC).

Media Composition Analysis

Cell culture media samples were analyzed using the Vi-CELL MetaFLEX™ bioanalyte analyzer (Beckman Coulter, Inc.) to evaluate pH, partial pressure levels of oxygen and carbon dioxide (pO_2 and pCO_2), glucose, lactate and electrolytes.

RESULTS

Flexible culture volumes via Culture Clamps

Prototype culture clamps were used to create defined cell culture compartments for VueLife® bags. This facilitates the use of the same bag with different fill volumes, providing flexibility in the volumes used for cell expansion while maintaining an even fill layer at 1 cm height. As detailed in Table 1, the culture clamps were placed at the first quarter of the length of the bag and then filled with 25 mL or 50 mL of culture medium.

Initial Clamp Position	Initial Fill Volume	Clamp Position Day 3	Fill Volume Day 3	Clamp Position Day 5	Fill Volume Day 5
quarter	25 ml 50 ml	middle	100 ml 200 ml	removed	500 ml

Table 1. Placement of culture clamps and fill volumes during T cell expansion in 200-HP bags.

T cell density and aggregation in "HP" bags

Cell density was maintained throughout the duration of the experiment at $1x10^6$ cells/mL. We found T cell expansion to be optimal when seeded at ~0.5×10^6 cells/mL, and that dilution of cells < 0.3×10^6 cells/mL was often detrimental to efficient expansion in bags.

The culture bags were visually examined every 2-3 days, cell aggregates dispersed by manually homogenizing the culture medium, and culture samples collected to determine cell growth and medium composition. It was found that if cell aggregation could easily be dispersed, the cell yield and health were overall improved compared to strongly aggregated cell clusters.

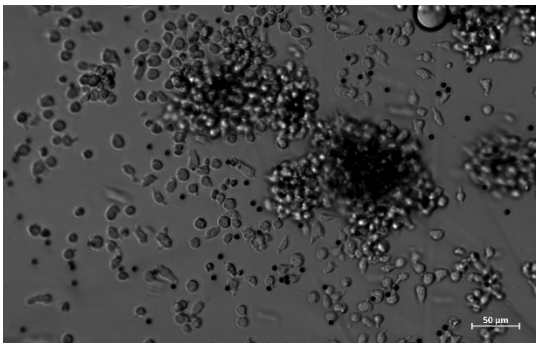

Figure 2. Microphotograph of T cells cultured in 200-HP bags after 7 days of culture. Scale bar indicates 50um.

T cell expansion in 200-HP bags

To directly compare the performance of VueLife® "HP" Series Bags with their "C" Series counterpart, we expanded isolated $CD4^+/CD8^+$ T cells from the same starting population in 200-HP and 197-C bags for 10 days. As illustrated in Figure 3, T cell expansion typically increased ~2-fold in VueLife® 200-HP bags compared to the 197-C bags for

each donor-derived T cell population. Cell viability (Figure 4) was comparable for T cells cultures in both bags, and remained at > 85% at time of harvest.

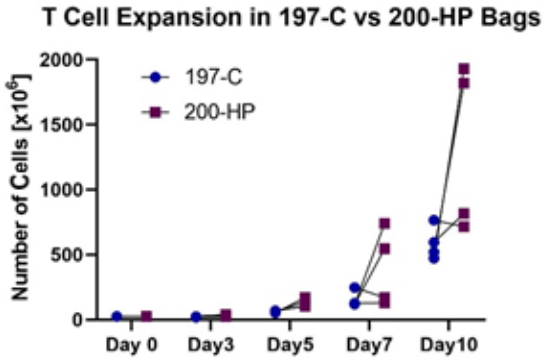

Figure 3. Number of T cells produced in VueLife® 200-HP bags vs 197-C bags. T cells were isolated from 4 individual donors and seeded into 197-C and 200-HP bags for direct comparison. The lines connect the results observed for the same donor.

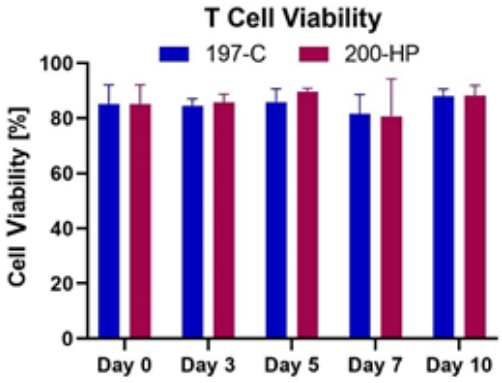

Figure 4. T cell viability in 200-HP and 197-C bags at the indicated time of culture. Results are mean ± SD values from n=4 experiments.

After the initial seeding phase, T cells require consistent dilution and adjustment of culture volumes with fresh medium to maintain logarithmic expansion towards clinically-relevant numbers. To understand the impact of cell dilution and addition of media at early stages of culture on the ultimately achieved cell count, we tested different cell dilutions at day 3 and 5 of culture. As shown in Figure 5, T cell expansion was overall higher if culture volumes were gradually increased (day 3/100 mL -> day 5/200-300 mL) compared to a single large increase (day 3/100 mL-> day 5/500 mL) in 200-HP bags. Cell viability was also generally improved in systems cultured at more incremental increases in culture volumes.

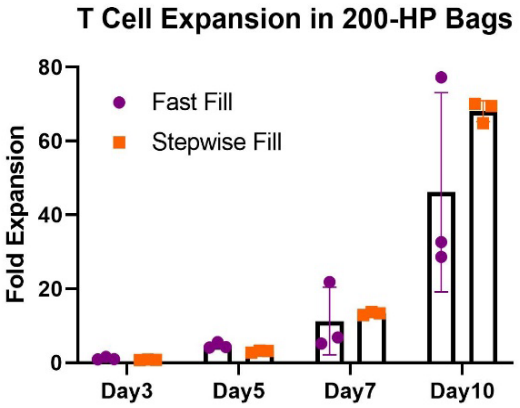

Figure 5. Impact on media addition protocol on T cell yield. T cells from n=3 donors were cultured in 200-HP bags for 10 days. In the "Fast Fill" method, media volumes were increased quickly, from 25 mL (Start/Day 0) to 100 mL (Day 3) to 500 mL (Day 5). In the "Stepwise Fill" method, we increased volumes slower, from 25 mL (Start/Day 0) to 50 mL (Day 3) to 200 mL (Day 5) to 500 mL (Day 7). Shown are mean ± SD values of fold increase results (vs Day 0).

T cell activation and phenotyping

After enrichment of $CD4^+/CD8^+$ T cells from the Leukopak™ and starting material, the isolated cells were activated with Dynabeads™ at a 1:1 ratio. Cell activation could be tracked via changes in cell diameter and expression of CD25 during culture, as shown in Figure 6.

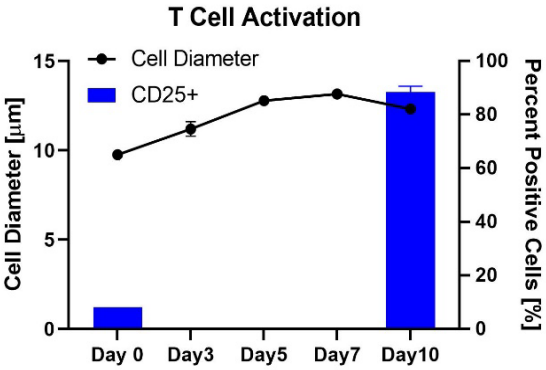

Figure 6. T Cell Activation in 200-HP bags. Show are changes in cell diameter and expression of CD25 on T cells cultured in 200-HP bags for 10 days. Shown is a representative example with results from one donor.

At the start (Day 0) and after 10 days of expansion, the cultured cell population was analyzed for the expression of canonical T cell markers CD3, CD4 and CD8, as well as T cell subset composition via CD45RO and CD62L. As shown in Figure 7, the composition of the T cell population changed during culture, but remained comparable between 197-C and 200-HP bags, indicating that the produced T cell population is at least partially driven by culture media composition and microenvironment.

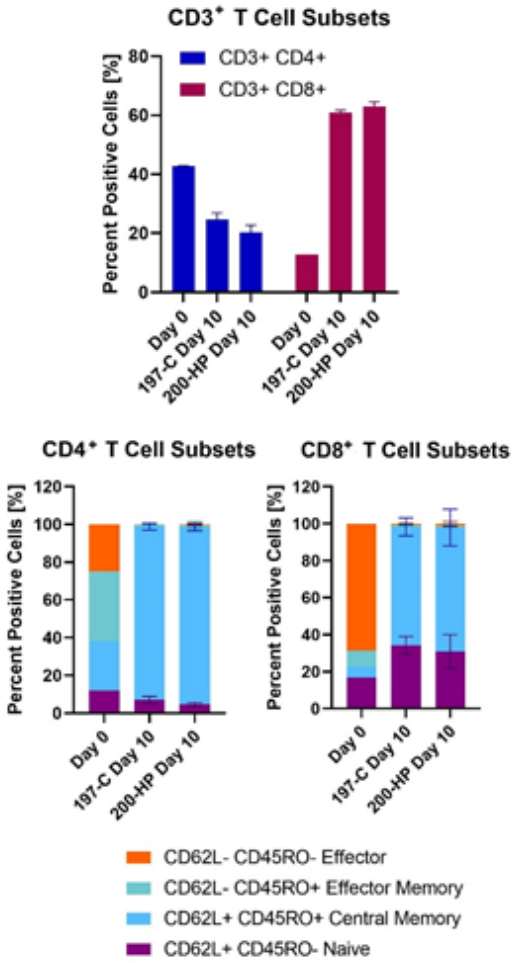

Figure 7. T Cell Phenotype before and after culture in VueLife® "C" and "HP" Series Bags. Shown are mean ± SD values of percent positive cells [%] expressing the indicated marker(s) relative to unstained controls.

Cell metabolic analysis

Samples of cell culture media in VueLife® "C" and "HP" bags were collected before any adjustment in cell numbers and analyzed for concentrations of metabolic parameters, including glucose, lactate, partial pressure of oxygen (pO_2) and carbon dioxide (pCO_2). As expected the concentrations of glucose and lactate were inversely correlated. As shown in Figure 8 below, we also observed a correlation between glucose levels and in cell expansion, suggesting that monitoring of glucose consumption may be a useful marker to help assess cell performance in culture bags.

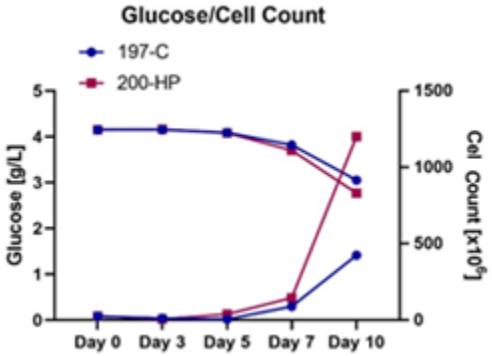

Figure 8. Cell count and glucose concentration. In our experiments, we found a correlation between consumption of glucose and increase in cell numbers. Shown are results from a representative experiment.

CONCLUSIONS

The results of this study highlight the improved performance of Saint-Gobain Life Sciences' novel VueLife® "HP" Series Bags for the expansion of human T cells towards clinical use in Cell and Gene Therapy applications.

The protocol and results described here are intended to provide an example to illustrate the performance of VueLife® HP bags with the products and culture parameters used in this setting. Many variables can affect T cell expansion, including starting cell populations (T cell subsets), cytokines, and growth factors. Thus, users will need to investigate the culture conditions that will provide the optimal result with their specific protocols.

Please contact us if you have any questions related to the information presented here. We are continuously working to improve the use of our products.

ABOUT

SAINT-GOBAIN

Saint-Gobain Life Sciences is proud to take part in providing solutions for a multitude of cell therapy applications while collaborating with customers and industry partners to develop custom disposables, often for integration into automated systems. Through our material science expertise as well as our deep experience in bringing manufacturing technologies to scale, we are uniquely positioned to offer solutions to the numerous challenges faced by cell therapy manufacturers today.