Using the AppliFlex ST to Investigate the Effect of CO_2 Concentration on the Expansion of T-cells

Wiegmann, V.[1], Amini, A.[1], Bernal, C.[2]. [1]Department of Biochemical Engineering, UCL, Bernard Katz Building, WC1H 0AH London, UK [2]Applikon Biotechnology BV, Heertjeslaan 2, 2629 JG, Delft, The Netherlands

Introduction

In recent years, adoptive T-cell therapy (ACT) has emerged as a promising way to treat systemic cancers such as Acute Lymphoblastic Leukemia. However, robustness and reproducibility of the manufacturing process remain challenging and it is therefore pivotal to understand the effect that cell culture conditions have on the expansion and differentiation of T-cells. This work investigates the effect of CO_2 on the expansion of T-cells using the bioreactor AppliFlex ST due the advantages of this single-use system such as lower initial investments, faster setup and reduced cross-contamination risks. The influence of CO_2 is of particular interest with regards to potential allogeneic T-cell therapies and the associated cultivation at the large scale, where CO_2-removal may become insufficient. Several studies have demonstrated for other mammalian cell types that elevated concentrations of dissolved CO_2 can severely affect the bioprocess (e.g. Brunner et al., 2018, Nguyen Dang et al., 2019), but so far no such studies exist for the expansion of T-cells.

Materials & Methods

- All experiments were performed in AppliFlex ST single-use bioreactors with a nominal volume of 500 mL (Figure 1) and a marine impeller.
- For measurements and control of the parameters the my-control was used. The culture conditions are described in Table 1.
- All conditions were replicated with n ≥ 2 donors.
- Bioreactors were supplied with gas mixes of 5%, 10%, and 20% CO_2 balanced with N_2 or O_2.
- Viable cell concentration and viability were determined using a NucleoCounter NC 3000 (ChemoMetec A/S, Denmark) using Via 1 Cassettes (ChemoMetec A/S, Denmark).
- Concentrations of nutrients and metabolites were measured using a Bioprofile Flex (Nova Biomedical, USA).

Cell Culture Conditions

Working volume	150 - 300 mL
Gas flow rate	1.0 vvm Overlay
Control of pH	7.2 with CO_2 ↓, 250 mM bicarbonate buffer ↑
Control of DO	25% with Air ↑, N_2 ↓
Mode of operation	Fed-Batch (+100% of initial working volume)
Temperature	37°C
Seeding density	0.4E6 cells mL^{-1}
Agitation	200 rpm
Medium	X-Vivo 15 + 5% FBS + 1% Pluronic F68

Table 1 | Culture Conditions for T-cells in the AppliFlex ST

Figure 1 | AppliFlex ST (Getinge)

Results & Discussion

- Cell growth is near-identical between donors at lower concentrations of CO_2. Only cells of Donor 14 appeared to be affected by an increased concentration of CO_2. In this case, growth stalled after 3 days of cultivation and the viability slowly decreased (Figure 2 and Table 2).
- Interestingly, cells of Donor 14 grown with 20% CO_2 showed an increased specific generation of lactate and consumption of glucose compared to those cells grown at the lower concentrations of CO_2. An involvement of the pCO_2 on the lactate metabolism has previously been demonstrated for other mammalian cells (Brunner et al., 2018).
- Using the outlined fed-batch process, the glucose concentration was maintained above depletion level and the concentration of lactate and ammonia were maintained below 6 g/L and 3 mmol/L, respectively. Some conditions were potentially glutamine limited in later stages of the cultivation. Further optimisation of the process could include enhanced supplementation with glutamine.

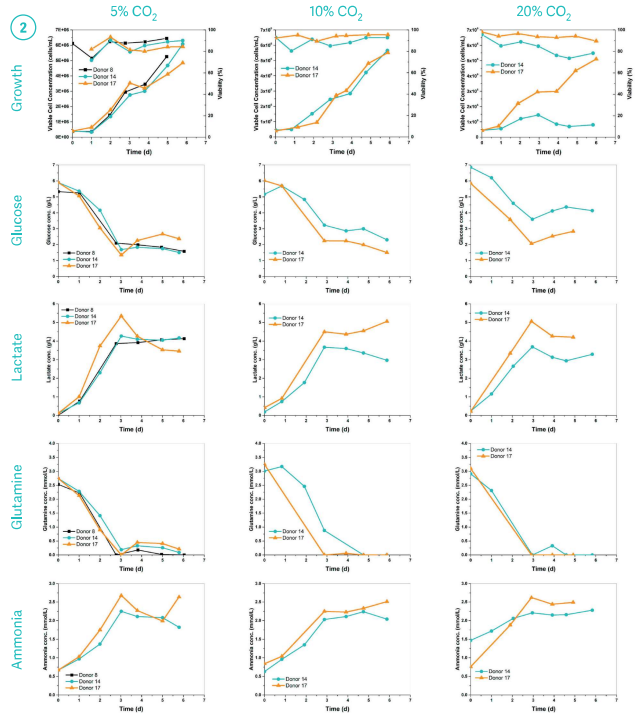


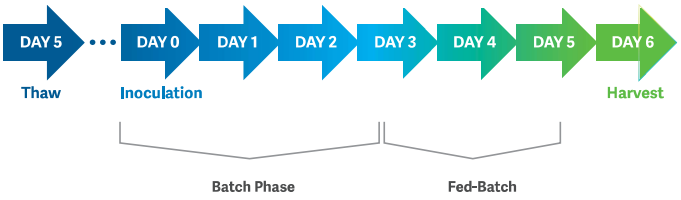
Figure 2 | Growth and metabolism of primary T-cells expanded using the AppliFlex ST single-use vessel. All inflowing gases contained either 5%, 10%, or 20% CO_2.

Fold Expansion

	Donor 8	Donor 14	Donor 17
5% CO_2	27,8	30,4	24,3
10% CO_2	x	24,8	27,9
20% CO_2	x	3,6	23,2

Table 2 | Final fold expansion of T-cells in the AppliFlex ST under different CO^2 concentrations

Process Flow



Conclusions

- The AppliFlex ST 500 mL single-use vessels were successfully employed for the expansion of primary T-cells.
- Different concentrations of CO_2 in the inflowing gas were tested for their effect on growth and metabolism of primary T-cells. Concentrations of up to 10% CO_2 were tolerated by all tested donor cells. At 20% CO_2 one of the tested donor cells stalled in growth and showed a change in metabolism, whereas cells of the other donor were unaffected.
- The results highlight that sensitivity to CO_2 toxicity is donor-dependent. With regards to large-scale manufacturing of allogeneic T-cell therapies, it is therefore critical that CO_2 resistance is considered during the cell line selection.

References

- Brunner M, Doppler P, Klein T, Herwig C, and Fricke J. Elevated pCO_2 affects the lactate metabolic shift in CHO cell culture processes. Eng. Life Sci. 18(3), 204–214 (2018).
- Nguyen Dang A, Mun M, Rose CM, et al. Interaction of cell culture process parameters for modulating mAb afucosylation. Biotechnol. Bioeng. bit.26908 (2019)

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